The American Journal of Human Genetics

Vol. 11 No. 3 *September 1959*

The Chromosome Constitution of a Human Phenotypic Intersex

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INTRODUCTION

SHORTLY AFTER the original description of sex chromatin (Barr and Bertram, 1949, see also Graham and Barr, 1952), application was made of the technique to the sexing of human hermaphrodites (Moore, Graham, and Barr, 1953). A great number of papers on this and similar subjects has appeared subsequently, in which the utility of the sex chromatin technique in diagnosing the genetic sex of many cell types has been demonstrated. To date, however, there have been very few investigations of human intersexes in which analysis of metaphase chromosomes has confirmed the results derived from sex chromatin determinations (Ford *et al.*, 1953; Jacobs and Strong, 1959; Chu and Giles, 1959).

Recently, a case of this type in a twelve-year-old individual has been investigated. As a result of clinical observations to be described below, it was believed that analysis of the chromosome complement of this individual might be valuable.

For the purposes of an investigation of another kind, a technique for the karyotype analysis of human peripheral leukocytes had been developed (Nowell *et al.*, 1958). Normally, mitosis is not observed in such cells *in vivo*. However, in our modification of the gradient culture method of Osgood and his colleagues (1955), high frequencies of mitosis had been observed. Results from the application of this technique as well as from observations on sex chromatin in the individual mentioned above will be described in this paper.

Case Report

Patient J. G., a Negro, was 12⅓ years old when first seen. The chief complaint was of swelling in the left breast, first noticed 3 or 4 days after the breast had been struck by a baseball approximately 4 months before the patient was first seen. Two months after the injury enlargement of the right breast was noticed (Fig. 1). No pain was associated with these mammary changes.

The past medical history did not seem relevant to the chief complaint, and the history of symptoms relating to specific systems did not suggest any irregularities.

Received April 21, 1959.

[1] Supported by a research grant from the American Cancer Society to Dr. Jack Schultz.

[2] Supported in part by Senior Research Fellowship SF-4 from the U. S. Public Health Service, and in part by grant C-3562 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

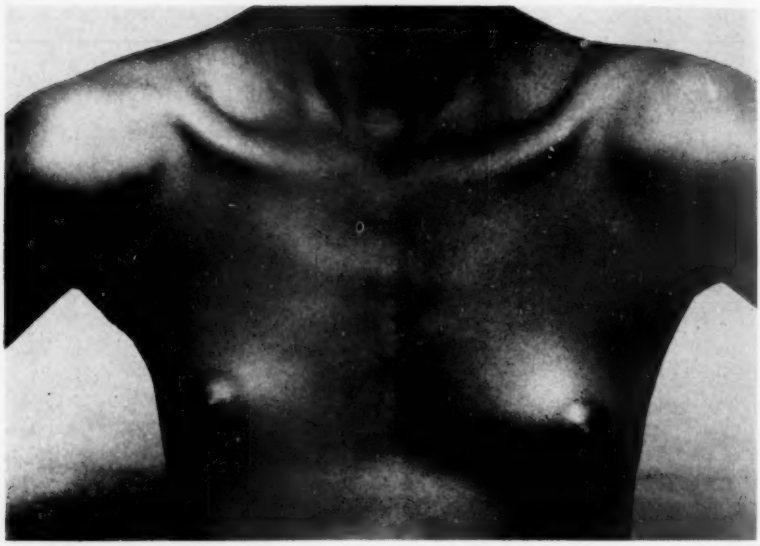

FIG. 1—Preoperative gynecomastia, pronounced on left side.

The family history was unobtainable, since the child had been abandoned by his parents and later adopted by foster parents.

The youth was a slender but well developed and well nourished individual. No abnormalities could be discovered except those relating to the breasts and to the contents of the right scrotum. The left breast was uniformly soft and enlarged to about 6 cm. in diameter with a central depth of approximately 3 cm. The right breast had a similar consistency, but the size approximated 3 cm. in diameter with a central depth of 2 cm. (Fig. 1). A clinical diagnosis of gynecomastia was made, and the possibility of a right testicular lesion noted. The penis was small, although within the normal size range and configuration for a male in early adolescence (Fig. 2). Hypospadias was not evident. The right scrotum contained a palpable ovoid mass, presumably testicle, with a tumor-like irregularity at the superior pole (Fig. 2, arrow). The nodule measured approximately 2 x 1 x 1 cm. The left scrotum contained a somewhat smaller ovoid structure judged to be testicle. The median raphe joining the scrotal sacs was complete and without suggestion of vaginal indentation (Fig. 6). The escutcheon was equivocally female. A small mass, possibly prostate gland, was palpated by rectal examination.

A left subcutaneous mastectomy was performed under general anaesthesia (Fig. 3). At the same time a biopsy specimen was taken from the mass presumed to be testicle in the right scrotal sac. Histopathological study of the left breast tissue resulted in a diagnosis of gynecomastia with duct hyperplasia and cyst formation. No acini were found in the breast tissue, however, the duct epithelium presented in some areas a minimal papillary cellular hyperplasia. The small biopsy specimen from the

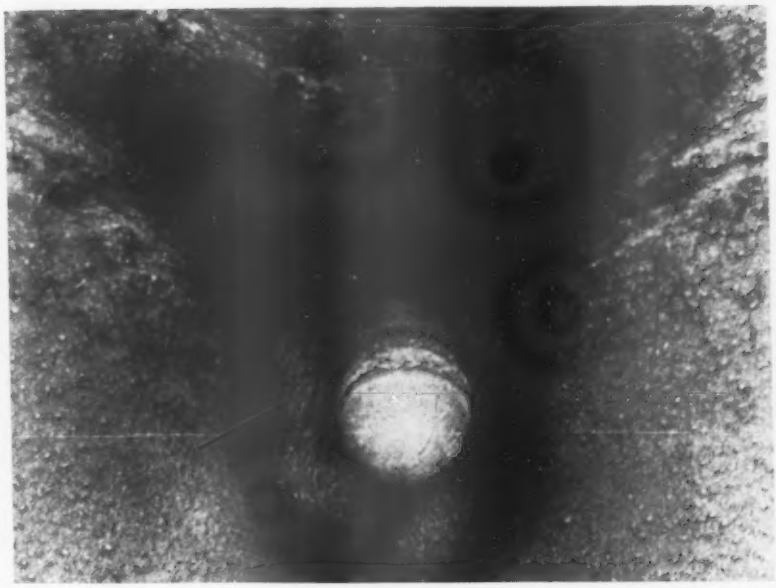

FIG. 2—External genitalia, preoperative. Arrow indicates site of tumor-like irregularity (see text).

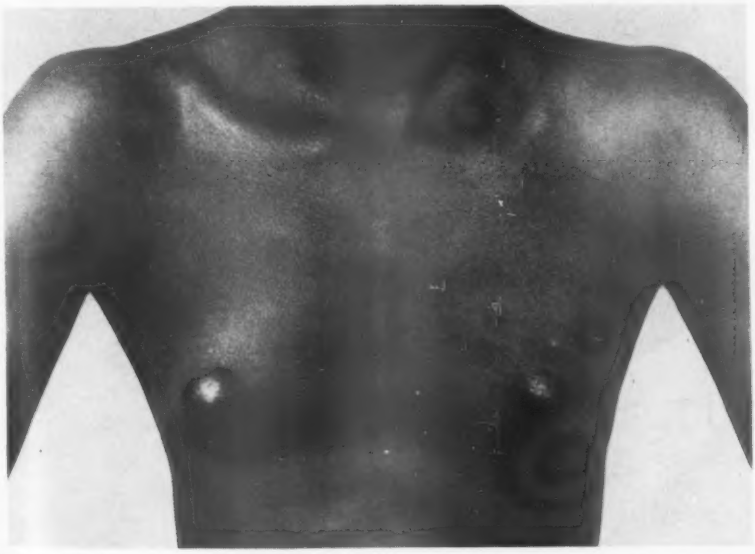

FIG. 3—Postoperative photograph. Note surgical scar on left side and development of gynecomastia on right side.

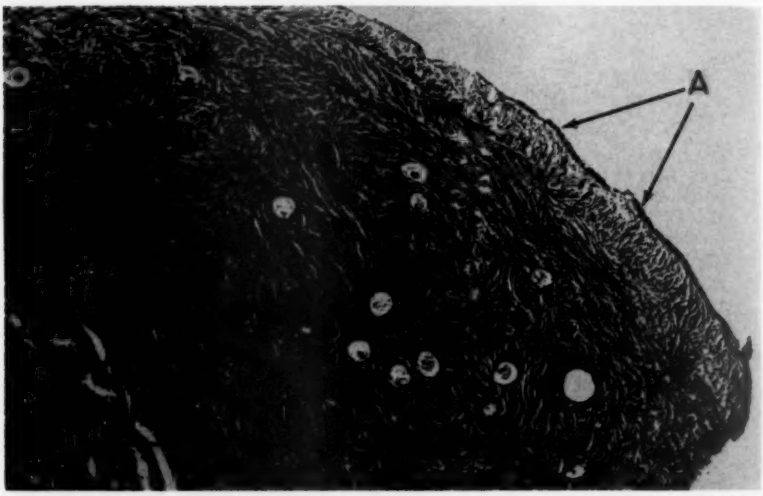

FIG. 4—Low power photomicrograph of biopsy specimen from mass originally presumed to be testicle in right scrotal sac. Note typical ovarian tissue with germinal epithelium (A), and scattered primary follicles in ovarian stroma. Hematoxylin-eosin. Scale indicates one millimeter.

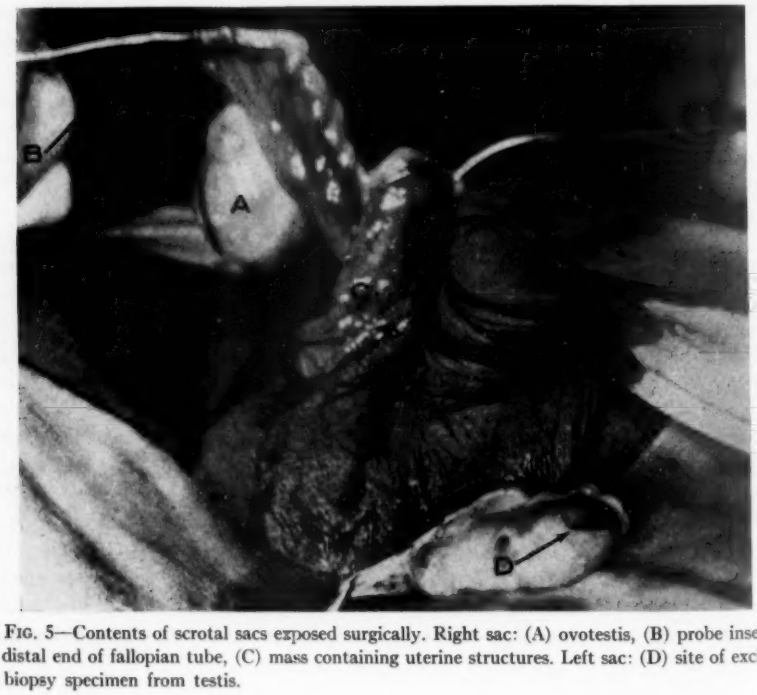

FIG. 5—Contents of scrotal sacs exposed surgically. Right sac: (A) ovotestis, (B) probe inserted in distal end of fallopian tube, (C) mass containing uterine structures. Left sac: (D) site of excision of biopsy specimen from testis.

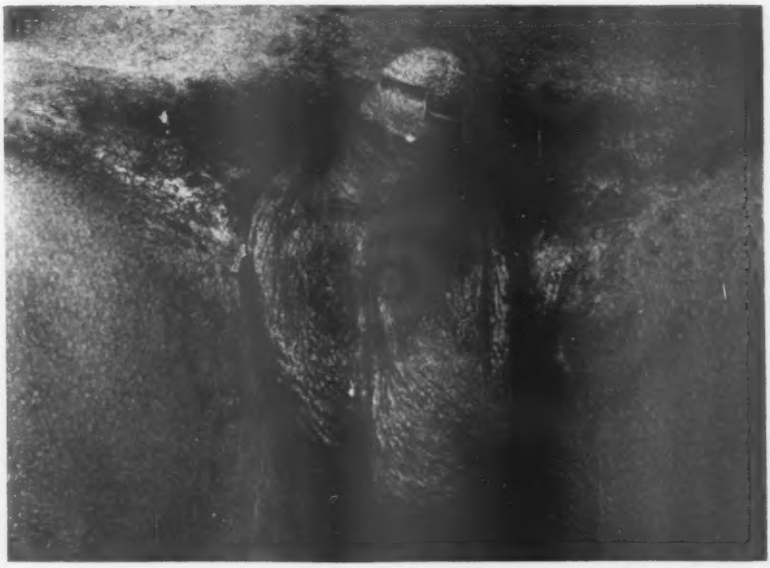

FIG. 6—External genitalia, postoperative. Note removal of contents of right scrotal sac. Note also that there is no evidence of hypospadias, and no suggestion of vaginal indentation of the median raphe.

contents of the right scrotum was recognized as *ovarian* tissue, with many primary follicles scattered in a typical ovarian stroma (Fig. 4).

Subsequently, investigations were made of the sex chromatin pattern in the peripheral blood neutrophils and in nuclei of various other cell types. The pattern in each instance was female. Most of the laboratory studies on the patient fell within normal limits.

Roentgenographic studies of the chest and osseous development brought to light no abnormalities. Studies of the sella turcica demonstrated a relatively small but otherwise normal structure. The intravenous urogram was normal.

Permission to perform a laparotomy could not be obtained from the foster parents, but their cooperation was gained in allowing the removal of the abnormal contents of the scrotal sacs. When the right scrotum was opened an almond shaped body measuring 3 x 2 x 1.5 cm. was found in the lower portion normally occupied by the testicle (Fig. 5, A). This structure was cystic and attached on one side by soft fibrotic tissue to a convoluted tubular structure 5 cm. long in its unextended state and 0.5 to 0.7 cm. in diameter (Fig. 5, B). That part of the tube associated with the ovoid cystic body resembled a fimbriated end. The opposite extremity was intimately attached to a resilient tissue of irregular outline (Fig. 5, C). This region of tissue included one more or less solid mass (about 1.5 cm. in its greatest dimension), occupying the superior part of the scrotal sac just below the right external inguinal ring. The impression gained from the gross appearance was that the tissues represented ovary, fallopian tube and rudimentary uterine tissue.

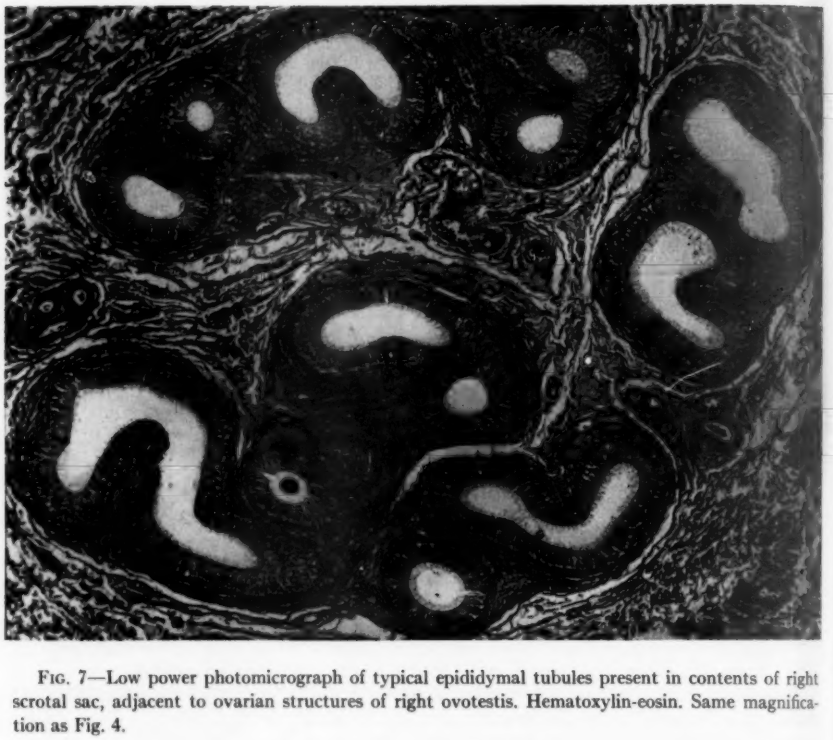

FIG. 7—Low power photomicrograph of typical epididymal tubules present in contents of right scrotal sac, adjacent to ovarian structures of right ovotestis. Hematoxylin-eosin. Same magnification as Fig. 4.

On the basis of the patient's male anatomical characteristics, rearing and psychological orientation, all of the right scrotal contents were removed, after it had been determined that the left scrotum contained a well formed but small testicle and epididymis. Biopsy specimens were taken from these structures (Fig. 5, D).

Histological studies of the tissues confirmed the impressions given by the gross surgical anatomy. The ovarian tissue (Fig. 4) contained primordial follicles, follicular cysts, corpora albicantes and ovarian stroma. The ovarian stroma also contained structures resembling testicular tissue, including atrophic seminiferous tubules and interstitial cells (Leydig cells), the presence of which indicated that this gonad was an ovotestis. Other structures were quite evidently epididymis (Fig. 7) and a tubule with villi, judged to represent a fallopian tube, although in the latter some resemblance to a fetal vas deferens must be admitted. The resilient tissue at the upper end of the convoluted tube in the superior part of the right scrotum was histologically uterine, with a glandular mucosa suggesting endometrium. The endometrial stroma was not well represented. Biopsy specimens from the structures in the left scrotal sac proved them to be testicle with atrophic or hypoplastic seminiferous tubules (Fig. 8) and an associated epididymis. There was no evidence of spermatogenesis. These

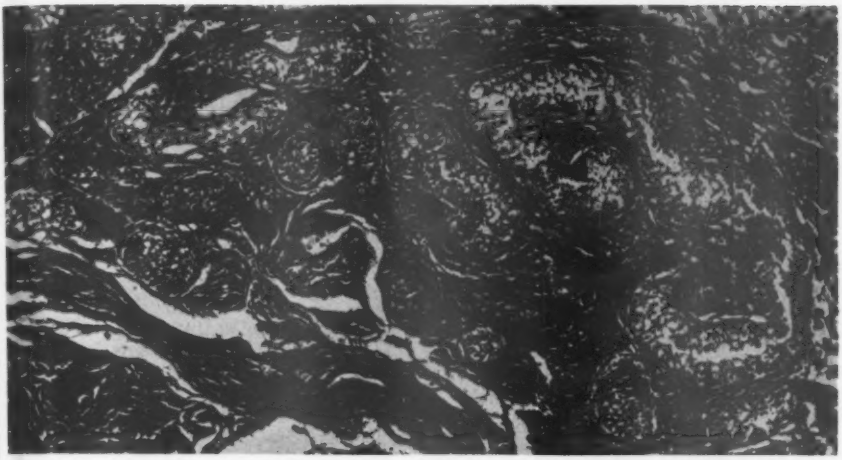

FIG. 8—Low power photomicrograph of biopsy specimen from left testis. Note atrophic seminiferous tubules. Hematoxylin-eosin. Same magnification as Fig. 4.

observations, made from hematoxylin-eosin stained sections of formalin-fixed tissues, established the diagnosis of phenotypic intersex.

Hormonal assays made on the patient's urine, preoperatively and postoperatively, proved to be significant. Before the removal of the ovotestis and related tissues from the right scrotal sac, the FSH urine gonadotropins were less than 6 mouse units per 24 hours, whereas a level of 96 mouse units per 24 hours was reached following the removal. This increase in pituitary activity was to be expected with a decrease in female gonadal tissue. The urine estrogens were first assayed at a level of 24 mouse units per 24 hours, but following the operation the level fell to 12 mouse units per 24 hours. This change is readily understandable, since the surgery deleted much estrogen elaborating tissue (evidenced by the sections, which included ovarian follicles, corpus luteum and stroma as well as other structures of female internal genitalia). The urine 17-ketosteroids were 6.3 mg/24 hours preoperatively and 8.5 mg/24 hours postoperatively.

CYTOLOGICAL STUDIES

Sex chromatin: Preparations for this purpose were of three types. Smears of cells scraped from the buccal mucosa were fixed in ether-alcohol (1:1) and stained by the Feulgen-fast green method (Fig. 9). Peripheral blood smears used for observations of "drumsticks" in polymorphonuclear leukocytes (Davidson and Smith, 1954) were stained with Wright's stain (Fig. 10). Sections from formalin-fixed tissue were prepared specifically for investigation of sex chromatin by the Feulgen method with fast-green counterstaining (Figs. 11–17).

Preparation of mitotic cells: Our modification of the method of Osgood and his

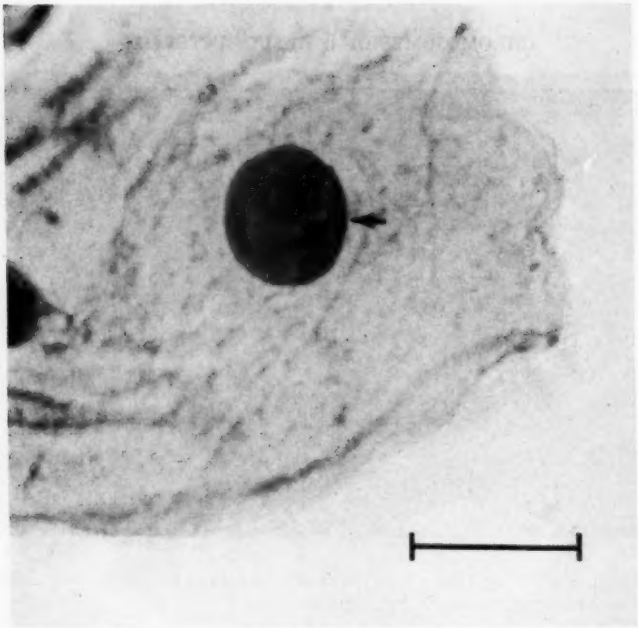

FIG. 9—Squamous epithelial cells in smear from buccal mucosa. Arrow indicates sex chromatin. Feulgen-fast green, 44× dry objective. Scale 10 μ.

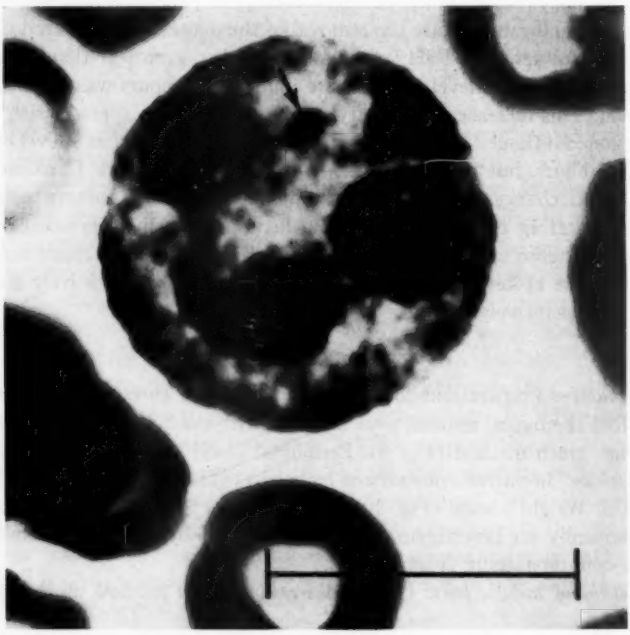

FIG. 10—Polymorphonuclear neutrophil from peripheral blood smear. Arrow indicates typical "drumstick," characteristic of females. Wright's stain, 90× oil immersion objective. Scale 10 μ.

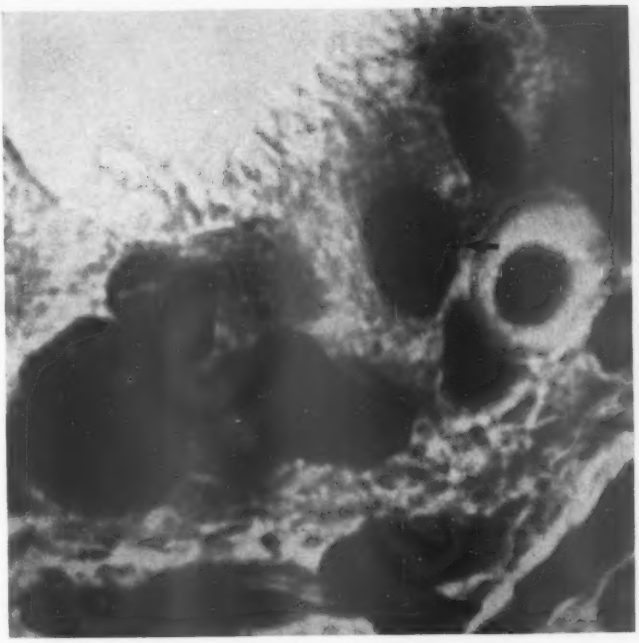

FIG. 11—Portion of ciliated epithelial lining of fallopian tube. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

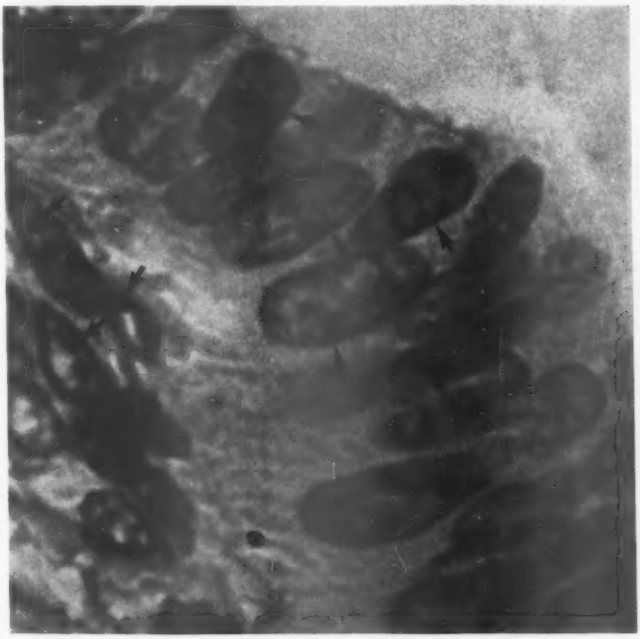

FIG. 12—Portion of endometrial epithelium with subepithelial fibrocytes. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

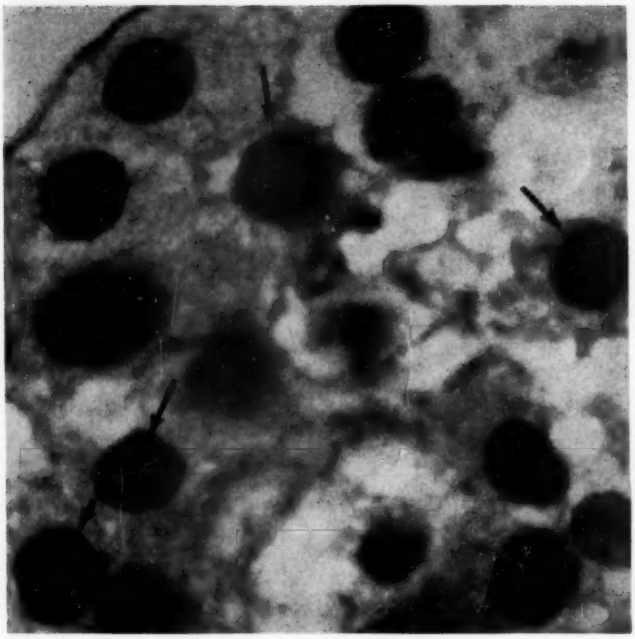

FIG. 13—Portion of corpus luteum from ovotestis. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

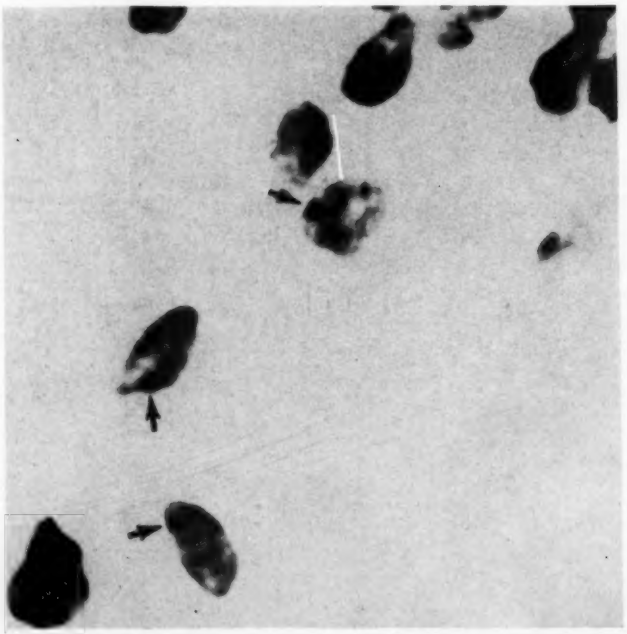

FIG. 14—Cells within atrophic seminiferous tubule of left testis. Note pycnotic character of the nuclei. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

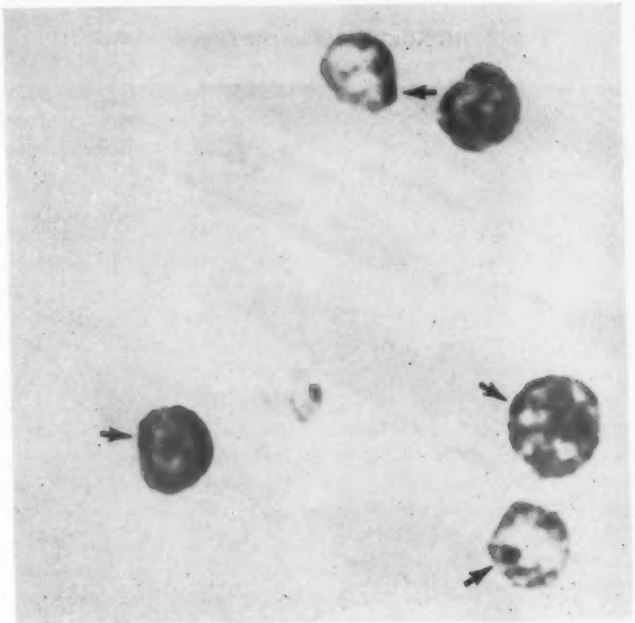

FIG. 15—Interstitial cells of left testis. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

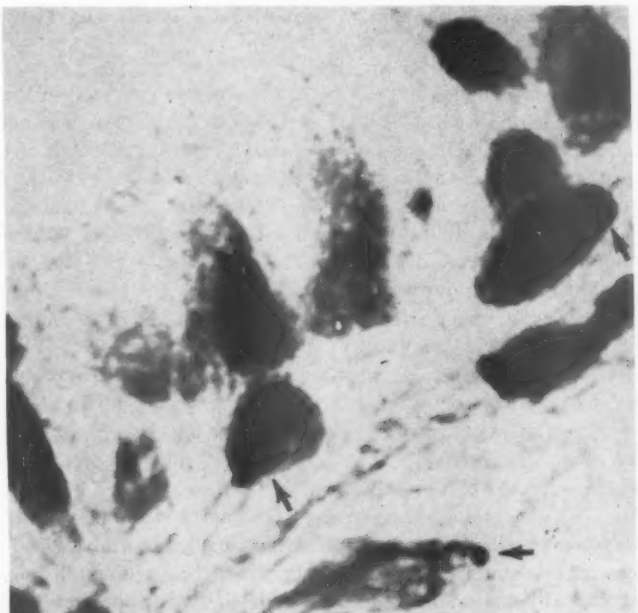

FIG. 16—Epithelium lining epididymal tubule. Arrows indicate sex chromatin. Note also sex chromatin in fibrocyte of supporting connective tissue (arrow nearest to lower right corner of the figure). Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

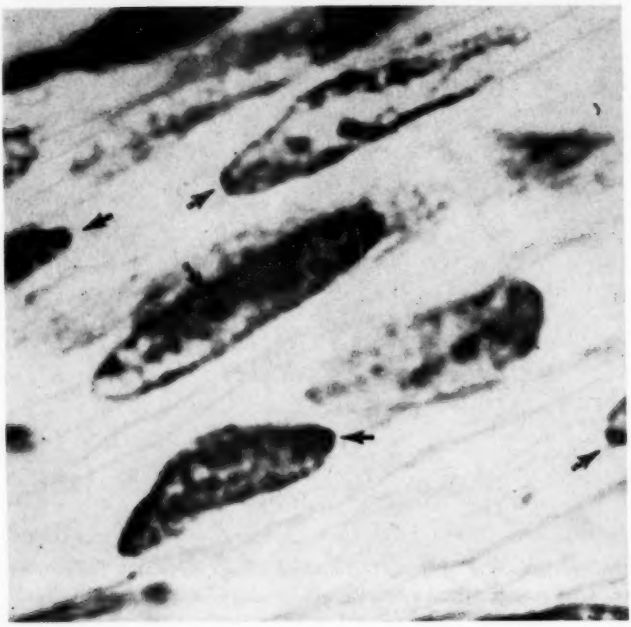

FIG. 17—Smooth muscle of fallopian tube. Arrows indicate sex chromatin. Feulgen-fast green, 44× dry objective. Same magnification as Fig. 9.

colleagues (1955) for the culturing of human leukocytes consisted in the following. To a volume of 15 to 30 ml. of heparinized venous blood there was added phytohemagglutinin (Difco) in the proportion of 5 to 6 drops per 10 ml. of blood. This mixture was allowed to stand in ice water for 30 to 60 minutes, after which sedimentation of erythrocytes was accomplished by centrifugation at 350 rpm for 10 minutes at 5°C. The supernatant, containing the leukocytes, was then pipetted off and the concentration of cells determined. The leukocyte suspension was then centrifuged at 2000 rpm for 3 minutes and the cells resuspended in a medium consisting of 10 to 20% normal human plasma in TC-199 (Difco) to which penicillin and streptomycin had been added. The initial concentration of cells was 1 to 2 $\times$ 10^6/ml. of culture. Culture volumes varied from 10 to 30 ml. and were planted in bottles of dimensions such that the depth of each culture was 3 to 4 cm. In all cases, one or two small glass slides were placed in the culture at an angle of approximately 60° from the vertical. Cells originally in suspension settled out and grew on the slides as well as on the sides and bottom of the culture bottles.

The dividing leukocytes are thought to be of monocytic and possibly lymphocytic origin in cultures of cells from individuals not having demonstrable blood anomalies. Our reasons for this statement are as follows. First, the dividing cells in our culture are morphologically too undifferentiated for classification; however, when periodic

addition of fresh serum is stopped and mitosis ceases, the entire population differentiates into monocytes, macrophages, and multinucleated giant cells. No cells resembling mature granulocytes or small lymphocytes are formed. Secondly, autoradiographic studies on the incorporation of tritium labeled thymidine into human peripheral leukocytes *in vitro* (Bond *et al.*, 1958) indicate that DNA synthesis occurs only in monocytes or large and medium-sized lymphocytes. Such observations support the interpretations of earlier workers in the field, who agreed generally that the nongranular leukocytes (*i.e.*, monocytes and perhaps lymphocytes) were the only cell types capable of surviving for more than a few days in culture (see references cited by Bloom, 1938).

Examination of cells growing on the slides has shown that, in the case of normal human beings, optimal mitotic activity can be expected after 3 to 4 days at 37°C. For the purposes of chromosome cytology, the cultures were terminated at this time. To accumulate large numbers of cells at metaphase, colchicine was added in a 1×10^{-7} M concentration at an interval of from 17 to 19 hours prior to termination. Cells were harvested from the culture bottles by shaking and decanting into centrifuge tubes, after which they were concentrated by centrifugal sedimentation, washed once with Earle's solution (Difco), and pretreated by incubation for 20 minutes at 37°C. in modified Niu-Twitty solution (Hungerford and DiBerardino, 1958), which is hypotonic for mammalian cells. Fixation and staining in 60% acetic orcein followed

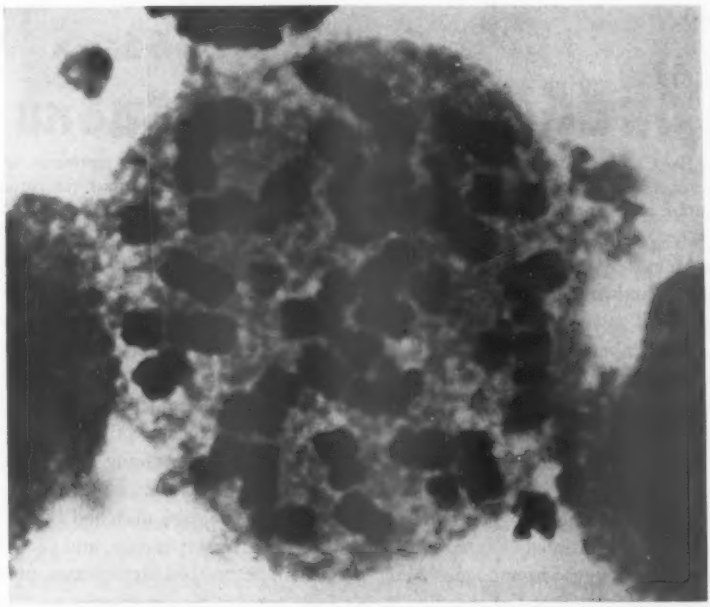

FIG. 18—Diploid metaphase plate ($2n = 46$) from peripheral leukocyte in short-term culture. Acetic orcein squash preparation, 90× oil immersion objective.

FIG. 19—Chromosome analysis of metaphase plate shown in Fig. 18. Scale represents 10 μ. The absence of a heteromorphic sex chromosome pair in the idiogram characterizes the chromosome constitution of the human female.

pretreatment. Squash preparations were made on unsiliconed slides with siliconed coverslips, and made permanent by the dry ice method.

Cytological analysis of metaphase chromosomes: Metaphase plates judged not to have been broken during squashing (Hungerford, 1955) were recorded by means of high resolution photomicrographs. Prints of these were made at 5320× magnification. Although approximately ¾ of this represents empty magnification, it provides a convenient size for subsequent steps in the method. Tracings of prints were checked and corrected by direct microscopic observation. A second tracing was made from the finished analysis of the metaphase plate and from this an idiogram was constructed. Usually, homologous pairs of chromosomes could be matched by means of two sets of observations: (1) characteristic chromosome morphology, and (2) a degree of loose association of homologues which persists in pretreated metaphases, providing that the organization of the spindle has not been too drastically disoriented. A study of the latter phenomenon has been under way at the Institute for Cancer Research for some years, and it is intended that details be reported elsewhere.

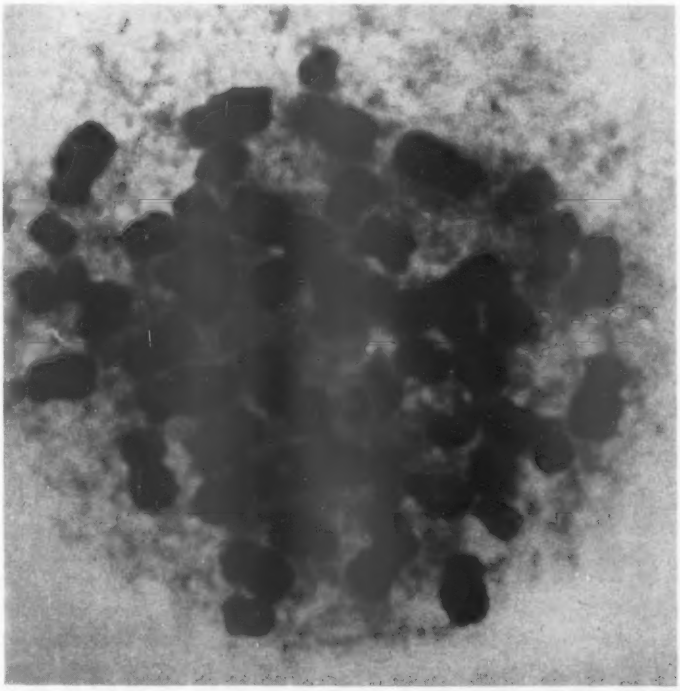

FIG. 20—Another diploid metaphase plate ($2n = 46$) from peripheral leukocyte in short-term culture. Acetic orcein squash preparation, 90× oil immersion objective.

The chromosomes have been classified (Figs. 19, 21) into three groups, according to centromere position: median (top row, seven pairs), subterminal (middle row, six pairs), and submedian (bottom row, ten pairs). Within each group, the pairs have been arranged according to length. Comparison of Fig. 19 with Fig. 20 indicates that the order within groups of chromosome pairs arranged by this method is not always the same; *e.g.*, the second and third pairs of chromosomes with submedian centromeres are in opposite sequence. Relatively different degrees of chromosome condensation or the effects of squashing may be responsible.

RESULTS

Chromosome counts from well-spread, intact metaphases are given in Table 1. Each count was made from a free-hand sketch. Only those which could be counted to within an accuracy of plus or minus one chromosome, and which fell within the diploid range, or the range of endoreduplicated (Levan and Hauschka, 1953) tetraploids are included. Two metaphases which were not accurately countable, but were approximately tetraploid and did not show quadruplochromosomes, are not included in the table. In addition to these metaphases, there were present in low frequency

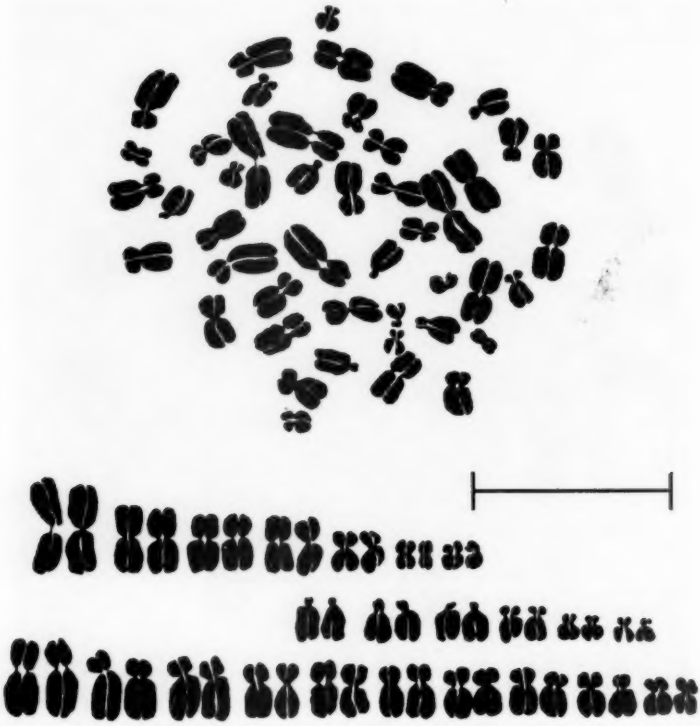

FIG. 21—Chromosome analysis of metaphase plate shown in Fig. 20. Scale represents 10 μ. Note again the absence of a heteromorphic sex chromosome pair.

hypoploid metaphases, which in all respects (Hungerford, 1955) appeared intact. Counts of 6, 10 (in three cells), 12, 13 $\pm$ 1, 15, 15 $\pm$ 1, 16 and 18 chromosomes were obtained; in addition there were several cells, not accurately countable, estimated counts from which ranged into the thirties. Whether such cells actually exist *in vivo* or represent an artifact of the culturing and/or preparative techniques is not at present known. Similar observations of metaphases with low chromosome numbers have been made in two other cases, a normal male and a normal female.

It is stressed that the counts in Table 1 are not to be interpreted as representative frequencies, since analyzable metaphases are not known to constitute a random sample of the metaphase population.

Chromosome analyses (Figs. 19, 21) of diploid metaphases (Figs. 18, 20) in this case are apparently identical with those from normal females. In no metaphase so analyzed (a total of five) was any indication of a hetermorphic sex-chromosome pair observed. Thus, the chromosomal sex of this cell type is female.

A list of the cell and tissue types in which the female sex chromatin pattern was observed is given in Table 2. All observations were made on Feulgen-fast green

TABLE 1.—METAPHASE CHROMOSOME COUNTS FROM LEUKOCYTES IN SHORT-TERM CULTURE

Chromosome number	45 ±1	46	46 ±1	47	47 ±1	46 ER	47 ER
Number of metaphases	3	34	4	1	6	2	1

Only counts accurate to within ±1 chromosome are included. ER denotes endoreduplication. See text for further details.

TABLE 2. CELL AND TISSUE TYPES IN WHICH FEMALE SEX CHROMATIN PATTERN WAS OBSERVED

1) Neutrophilic granulocytes in peripheral blood (Fig. 10)	Smear	Wright's stain			
2) Buccal squamous epithelium (Fig. 9)	"	Feulgen-fast green			
3) Epithelial cells of fallopian tube mucosa (Fig. 11)	Section	"	"	"	
4) " " " glands in endometrium (Fig. 12)	"	"	"	"	
5) Lutein " " corpus luteum from ovotestis (Fig. 13)	"	"	"	"	
6) Cells in cumulus oophorus of follicular cysts	"	"	"	"	
7) Glandular epithelium of breast	"	"	"	"	
8) Cells within seminiferous tubules (Fig. 14)	"	"	"	"	
9) Interstitial cell of testis (Leydig cells) (Fig. 15)	"	"	"	"	
10) Epithelial cells of epididymal mucosa (Fig. 16)	"	"	"	"	
11) Smooth muscle cells (Fig. 17)	"	"	"	"	
12) Supporting connective tissue cells (fibrocytes) (Figs. 12, 16)	"	"	"	"	

sections, except for those on the "drumsticks" (Davidson and Smith, 1954) in polymorphonuclear neutrophil, which were from smears stained with Wright's stain. The proportion of nuclei of interstitial cells of the left testis which were observed to have sex chromatin was 38% (in 6 μ sections). Sex-chromatin frequency was estimated to be approximately the same in other cell types. Thus, the diagnosis of chromatin sex in all of the tissues examined agreed with the sex determined by chromosome analysis.

An observation which seems noteworthy was made with regard to the sex chromatin in the nuclei of smooth muscle cells. In such nuclei, there is an obvious preferential localization of the sex chromatin at one or the other tip (Fig. 17). This phenomenon has to our knowledge not been described previously, and is under further investigation.

DISCUSSION

The classification of various types of abnormal sex differentiation, along with possible etiologies, has been thoroughly discussed by Danon and Sachs (1957) and Grumbach and Barr (1958), among others. Our case would seem to fit the clinical diagnosis of "true hermaphrodite," on the basis of both male and female gonadal tissue having been histologically identified.

Cases of human intersexuality for which chromosome analyses have been reported thus far in the literature number three. The first (Ford *et al.*, 1958), clinically a case of Klinefelter's syndrome (and having a female sex chromatin pattern), was diagnosed as female on the basis of five metaphases classified as female and one as probably female. The second case (Jacobs and Strong, 1959) was an apparent male with "... gonadal dysgenesis with gynaecomastia and small testes associated with poor

facial hair-growth and a high pitched voice." The individual's modal chromosome number proved to be 47, and on the basis of analysis of chromosome morphology in eight cells the possibility of an XXY sex-determining mechanism was proposed. The individual's sex-chromatin pattern was female. The third case (Chu and Giles, 1959) was a male pseudohermaphrodite, having a male sex-chromatin pattern. Chromosome determinations from nine cells, derived from thyroid biopsy material grown *in vitro*, showed 8 to have 46 chromosomes and one to have 47. The individual was XY.

It is not possible from the data at hand to resolve the genetic possibilities which might be involved in the case which has been described here. However, several such possibilities definitely may be ruled out. From the karyotype analyses of peripheral leukocytes and the sex chromatin constitution of several cell types, it may be stated that this individual is neither XY nor XO with regard to sex chromosome constitution. The karyotype analyses also indicate that the individual is not XXY.

Of the three most probable types of mosaic condition which might have been expected, *viz.*, XX-XXY, XX-XY, and XX-XO, the latter two can almost certainly be ruled out in all of the tissues examined on the basis of the observed relatively high frequencies of nuclei with sex chromatin present (indicating the presence of two X chromosomes in such nuclei (Reitalu, 1957)). The first posibility, XX-XXY, was not resolved in our study, although Sachs and Danon (1956) have stated that a chromocenter attributable to the Y chromosome can be recognized in certain epidermal nuclei and Reitalu (1957) has observed Y heterochromatin in fetal liver nuclei. Indeed, such mosaicism need not have been present in the single cell type from which karyotype analyses were made. Another possibility is that a mosaic condition might have been obscured by selective effects exerted by the *in vitro* environment.

Unfortunately, since our investigation was begun subsequent to the removal of the abnormal contents of the scrotal sacs, it was necessary to make sex chromatin determinations from those tissues fixed for routine histopathological diagnosis. Thus, it was not possible for us to make use of the method of Sachs and Danon (1956), which is reported to allow discrimination between X and Y chromocenters.

At least two other possible interpretations cannot be ruled out in this case. One is the strong likelihood of a genetic reversal of sex somewhat similar to that effected by the autosomal recessive gene mutation, "transformer," described in *Drosophila melanogaster* by Sturtevant (1945). The phenotype of female flies homozygous for this gene is male; it has no effect on genetic males. Interrogation of the presumed mother of this individual revealed it to be an adopted child, and it was not possible to obtain information relevant to the establishment of a pedigree.

The second possibility which we were unable to evaluate is that of a hormonal or other metabolic influence exerted on the fetus *in utero*. It is not unlikely that information of this kind might have been obtained from the real mother of the individual or her medical records, had they been available.

Regardless of the obscure nature of the very early embryological events in this case, it is virtually certain that the male gross anatomy of the patient is due to the presence of testicular tissue during virtually the entire course of fetal development. It has been well established, on the basis of fetal castration experiments in mammals (summarized by Jost, 1953), that the presence of a male gonad is required during

fetal life for the differentiation of male genitalia. Castration of female fetuses does not suppress the differentiation of female genitalia. However, male fetuses castrated early develop as females, and those males castrated at progressively later times develop as intersexes with progressively increasing degrees of maleness. The case of the freemartin supports such evidence: the presence of a male gonad in the male member of dizygotic twins of opposite sex, sharing a common blood supply, affects the phenotype of the female member such that it acquires male characteristics, although its sex chromatin pattern (Moore, Graham, and Barr, 1957), and presumably its sex chromosome constitution, remain unchanged.

SUMMARY

The chromosome constitution and the sex chromatin pattern of cells from a human phenotypic intersex have been investigated. A case report is given which includes a description of the individual's gross anatomy, as well as endocrine studies, pre- and postoperative histological diagnoses, and surgical therapy. Although the external anatomy, including genitalia, was that of a male, histological examination revealed the presence of ovarian tissue, fallopian tube and endometrium, as well as testis. The modal chromosome number in metaphases of leukocytes derived from peripheral blood and after short-term culture was found to be diploid ($2n = 46$) and the sex chromosome constitution to be female (XX). The sex chromatin pattern of various other cell types was consistent with a genetic sex diagnosis of female. Convenient methods for the short-term *in vitro* cultivation of leukocytes from peripheral blood and for the analysis of their metaphase chromosomes are described. Some of the genetic and embryological factors which may be involved in this case are discussed briefly.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Miss Sarah Reifsnyder, Miss Rose Shapiro, and Miss Elizabeth Yoast for expert technical assistance.

We are indebted to Dr. A. E. Rakoff for the assay studies done in the Endocrinology Laboratory, Jefferson Medical College and Hospital, Philadelphia.

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ADDENDUM

Since the present paper was submitted for publication, several papers have appeared relating defects in sex differentiation to chromosomal changes. One of these

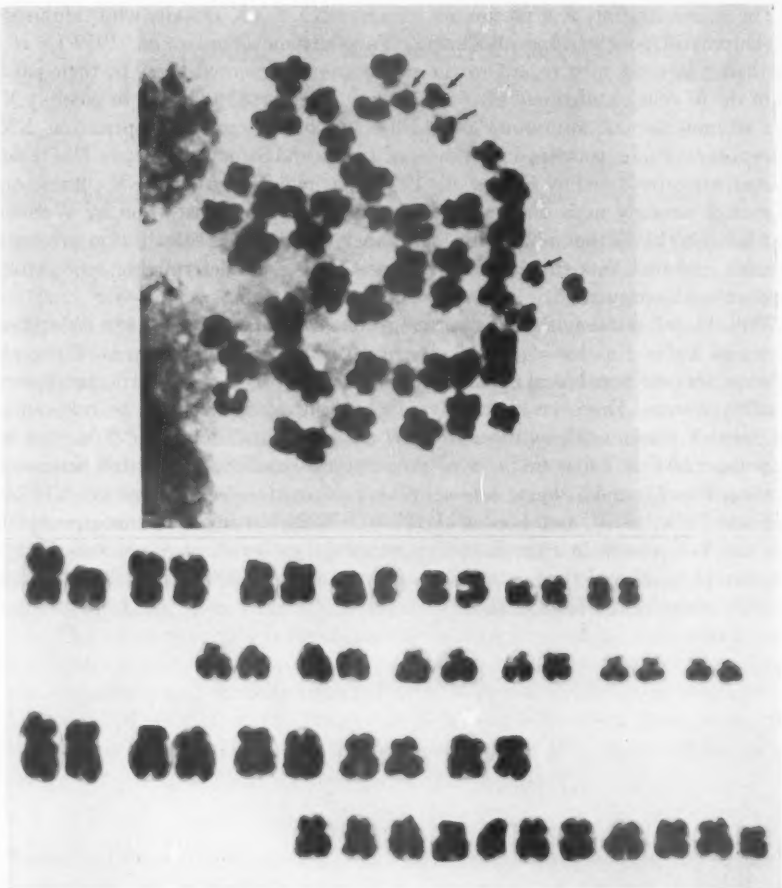

FIG. 22—Chromosome analysis of metaphase interpreted as having 47 chromosomes. Acetic orcein squash preparation, 90× oil immersion objective. Same magnification as Figs. 20 and 21. See text of Addendum for further details.

deals with a case of gonadal dysgenesis (Turner's syndrome), in which the majority of metaphases were observed to have 45 chromosomes and the sex chromosome constitution was interpreted as XO (Ford *et al.*, 1959 b—see also Fraccaro *et al.*, 1959). The individual was sex chromatin negative. Another deals with a sex chromatin positive case showing both mongolism and the Klinefelter syndrome (Ford *et al.*, 1959 a). In this case 48 chromosomes were present in all cells counted; the sex chromosome constitution was interpreted as XXY, and an additional small autosome was present, which is believed to be characteristic of mongolism (see also Jacobs *et al.*, 1959, Lejeune *et al.*, 1959 a and b).

The demonstration of a presumptive human XXY-XX mosaic, who exhibited a sex-chromatin positive case of Klinefelter's syndrome (Ford *et al.*, 1959 c), is of particular interest with regard to the observations presented here. In their paper, 44 of the 65 cells counted had 47 chromosomes (interpreted as XXY, or possibly XY and trisomic for one autosome) and 13 had 46 chromosomes (interpreted as XX). These observations, combined with those of Jacobs and Strong (1959) (see Discussion, above), are interpreted by Ford *et al.* (1959 c), as indicating that the Y chromosome in man is strongly male determining. The convincing demonstration by Welshons and Russell (1959) that mice with an XO sex chromosome constitution are fertile females, and thus that the mouse Y chromosome is male determining, lends strong support to this argument.

With this information in mind an attempt was made to analyze the two metaphases shown in Table 1 as having been counted at exactly 47 chromosomes. These two cells are not considered ideal for this purpose, and the origin of the extra chromosome remains obscure. However, the analyses were sufficiently accurate to rule out an unaltered Y chromosome as the additional one (Fig. 22). The normal Y in man has been described as being in the size range of the smallest acrocentric autosomes (arrows, Fig. 22) and as being acrocentric or telocentric—see Chu and Giles (1959), Tjio and Puck (1958), and Ford *et al.* (1958). Thus, barring the remote possibility that the Y is present in a translocation configuration, sex chromosome mosaicism is not present in this cell type, although as mentioned in the Discussion above it might exist elsewhere in the individual.

Are the MN Blood Groups Maintained By Selection?

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TAYLOR AND PRIOR (1939a) FOUND a significant excess of MN children from MN × MN matings, which they were convinced was not due to technical errors. Pooling their data with other studies, the proportion of MN children from intercrosses was 54.85%, the deviation from 50% being more than three times its standard error. Wiener (1943) attributed this excess to the use of incompletely absorbed sera, but his explanation was disputed by Haldane (1948), who could find no evidence for technical errors in the published data. Wiener's hypothesis was accepted by Race and Sanger (1954, p. 55), who remarked that "In our own Unit, since the war, we have tested 94 MN × MN matings with 218 children of which 119 or 54.6 per cent were MN; the excess was not significant ($\chi^2 = 1.8$ for 1 d.f.). We are now convinced that there is no true excess of MN children from this mating." Wiener (Wiener *et al.* 1953) published the results of his studies from 1949–52, which included 160 children from MN × MN matings, of whom 88 were MN. Pooled with the data of Race and Sanger cited above, there were 378 intercross children, of whom 207 were MN, or 54.8%. The excess over 50% is significant ($\chi^2 = 3.43$, P = .03 by a one-tailed test). Whatever the reason for an apparent excess of heterozygotes, it continues to operate in the most recent and carefully collected data. Wiener's tests to date (Wiener *et al.* 1953) give .508 ± .018 as the frequency of MN children from intercrosses, the standard error being large enough so that a substantial MN excess cannot be excluded.

THE SELECTION HYPOTHESIS

Taylor and Prior (1939b) showed that, in most population samples, the square of the number of MN individuals approximates four times the product of the M and N classes, and this fact may be used to detect gross errors in MN typing. The expected genotype frequencies are

M	N	MN
$m - mn\theta$	$n - mn\theta$	$2mn\theta$

where m and n are the frequencies of genes *M* and *N*, respectively, and $\theta = 1$ on the null hypothesis of random mating without selection or typing errors. If the gene frequencies are estimated from the sample, the maximum likelihood score for this hypothesis is $u_\theta = \{d\ln L/d\theta|_{m=\hat{m},\theta=1}\} = k(c^2 - 4ab)/(2a + c)(2b + c)$, where a, b, c are the observed numbers of types M, N, and MN, respectively, and

Received January 10, 1959.
Genetics paper No. 597.
This work was supported by a grant from the Rockefeller Foundation.

TABLE 1. ANALYSIS OF THE FREQUENCY OF MN PARENTS

	d. f.	m. s.	F	P
Deviation from hypothesis	1	3.18	1.96	.2–.1
Among sources	40	1.62	1.62	.01–.001

$\Sigma u = 144.5 \qquad \Sigma k = 6566$

$\theta^* = 1.022 \pm .016$

$a + b + c = k$. The variance of u_θ is $\{-d^2 \ln L/d\theta^2 |_{m=\hat{m},\theta=1}\} = k$ on the null hypothesis, so that for each sample u^2/k is the familiar χ^2 for testing goodness of fit for M, N, and MN frequencies. In a set of s samples, $\chi^2_1 = (\Sigma u)^2/\Sigma k$ tests the pooled deviation of θ from 1, and $\chi^2_{s-1} = \Sigma(u^2/k) - (\Sigma u)^2/\Sigma k$ tests heterogeneity of θ among samples.

The data consist of all European and American studies on the MN groups accessible to us (see references), in which families were reported separately (34 sources)* or the pooled segregation frequency was given for each mating type (9 sources). In two instances χ^2 for the above test is greater than 10; these results of Lattes and Garrasi (1932) and Dahr (1940) have been omitted from the sequel as presumably due to incompletely absorbed testing fluids, since other samples from the same populations show no such discrepancy.

There remain six samples with χ^2 between four and six, where a suspicion of technical errors might be entertained. The relevant values of $\chi = u/\sqrt{k}$ are: Landsteiner and Wiener (1941), + 2.00, Hirszfeld and Kostuch (1938), + 2.16; Zieve, Wiener, and Fries (1936), + 2.08; Wiener and Sonn (1943), − 2.20; Cotterman and Falls (1944), + 2.07; and Jameson, Lawler, and Renwick (1956), + 2.41. These may well be due to sampling error, especially since three of the studies were based on related families.

The analysis of all 41 sources remaining after exclusion of Lattes and Garrasi (1932) and Dahr (1940) is shown in Table 1. There is apparent heterogeneity among sources, but no significant excess of heterozygotes.

In most published studies on the MN groups, sterile matings were excluded and only tested children were recorded. Often family size was not complete at the time of testing. For these reasons and because of the possibility of reproductive compensation for fetal deaths in populations with voluntary restrictions on fertility, only gross reproductive differentials would be evident. Table 2 shows that, if the analysis of variance may be trusted in such skewed and heteroscedastic data, there are no significant and consistent differences in net fertility attributable to parental or progeny MN type, in the 33 studies where the number of tested children per family was reported.

Matsunaga and Itoh (1958) have published the only extensive study on MN blood groups and fertility. In their data there is no significant association between MN progeny and mortality, childless marriages, or the mean number of living children per woman. This does not exclude the possibility of differential fetal death in the

* One small Japanese study was included (Morton, Maloney, and Fujii, 1954). An extensive report on Japanese material will be published elsewhere (Chung, Matsunaga, and Morton, in preparation).

TABLE 2. ANALYSIS OF THE NUMBER OF TESTED CHILDREN PER FAMILY WITHIN SOURCES

	d. f.	m. s.	F	P
Mean regression on X_1	1	9.08	2.73	.2–.1
Among regressions on X_1	32	3.33	1.57	.05–.01
Reg. on X_2, X_3, X_4, X_5, after fitting X_1	4	0.32	0.15	.98–.95
Residual among families within sources	1980	2.12		

$b_1 = -.136 \pm .082$

$X_1 = 0$ for matings with no MN children expected
$= 1$ for matings with 50% MN children expected
$= 2$ for matings with 100% MN children expected
$X_2 = 1$ for M fathers, $= 0$ otherwise
$X_3 = 1$ for N fathers, $= 0$ otherwise
$X_4 = 1$ for M mothers, $= 0$ otherwise
$X_5 = 1$ for N mothers, $= 0$ otherwise

Mean no. of tested children per family = 2.86

first trimester, since most early abortions are probably not detected in marital histories.

If there is no differential mortality among MN groups in childhood and early maturity, and if technical errors occur with the same frequency in children and adults, the proportion of MN phenotypes should be the same in parents and children. We may arrange the observed and expected frequencies in a 2 x 2 contingency table as follows,

	observed numbers		expected proportion	
	MN	M or N	MN	M or N
parents	a	b	pr	$\theta p(1-r)/(1-p+p\theta)$
children	c	d	$(1-p)r$	$(1-p)(1-r)/(1-p+p\theta)$
total		$a+b+c+d=n$	r	$1-r$

where $\theta = E(bc/ad)$ is a parameter expressing the degree of association between blood type and generation, r is the frequency of MN individuals in the sample, and p is the expected frequency of parents in a sample with $\theta = 1$. Then $E(a) = pr$, $E(b) = \theta p(1-r)C$, $E(c) = (1-p)r$, and $E(d) = (1-p)(1-r)C$, where C is a constant such that $b + d = (1-r)n$. By the usual maximum likelihood methods we find $u = d\ln L/d\theta$ and $k = I_{\theta\theta} - I_{\theta p}^2/I_{pp}$, where the I's are the elements of the information matrix for θ and p, and both u and k are evaluated at the maximum likelihood estimate of p under the restriction that $\theta = 1$. On the hypothesis of no association ($\theta = 1$), the maximum likelihood score is $u = (bc-ad)/n$, with variance $k = (a+b)(c+d)(a+c)(b+d)/n^3$, if covariances between relatives are neglected. Table 3 gives the analysis of the 41 sources. There is no significant difference in the MN frequencies of parents and children.

Considering Tables 2 and 3 and the report of Matsunaga and Itoh, we conclude that there is no evidence of a relation between MN blood type and fertility, recognized fetal deaths, or survival to maturity. If there is a detectable effect of selection on the MN frequencies, it must operate on early and unrecognized fetal deaths, for which there may be complete reproductive replacement.

If we formulate the selection hypothesis in these terms, the only critical data come

TABLE 3. ANALYSIS OF THE MN FREQUENCIES OF PARENTS AND CHILDREN

	d. f.	m. s.	P
Deviation from hypothesis	1	.014	.95–.90
Among sources	40	1.259	.2–.1

$\Sigma u = -3.530 \quad \Sigma k = 911.615$

$\theta^* = .966 \pm .033$

from analysis of genetic segregation, and we can attribute any observed discrepancies to selection only if the possibility of technical errors can be excluded. We shall attempt to determine, first, whether an hypothesis of heterozygote advantage is consistent with the data, and secondly, whether the hypothesis of technical errors can equally well be maintained.

In the absence of fertility differentials, the most likely explanation of a balanced polymorphism is heterozygote advantage, the relative viability of M, N, and MN individuals being $1 - t$, $1 - ct$, and 1, respectively. In the world population, the gene frequency of *M* is .5556 (Taylor and Prior, 1939b), and if there were no reproductive compensation, the expected value of this gene frequency at equilibrium would be $c/(1 + c)$, which gives $c = 1.25$. The expected frequencies in segregating matings are as follows:

mating	children		
	M	N	MN
M × MN	$\frac{1 - t}{2 - t}$	0	$\frac{1}{2 - t}$
N × MN	0	$\frac{1 - ct}{2 - ct}$	$\frac{1}{2 - ct}$
MN × MN	$\frac{1 - t}{4 - t - ct}$	$\frac{1 - ct}{4 - t - ct}$	$\frac{2}{4 - t - ct}$

To analyze the data, we make use of the fact that the maximum likelihood score for a segregation frequency p is $u_p = r/pq - s/q$, with variance $k_{pp} = s/pq$, where r is the observed number of the class with frequency p, s is the total number in the sample, and $q = 1 - p$ (Morton, 1958). Since the score for t is $u_t = (dp/dt)u_p$, with variance $k_{tt} = (dp/dt)^2 k_{pp}$, we may carry out an analysis of t within and among mating types, assuming that $c = 1.25$. Intercrosses are partitioned into the M, N and M + N, MN segregations.

Table 4 gives the results for an initial estimate of $t = .0939$, which is close to the maximum likelihood estimate of $.0785 \pm .0195$. Several families with genetic exceptions (perhaps extramarital) have been omitted.

The significance of heterozygote excess is unquestionable ($P < .0002$). On the model of simple heterozygote advantage, the segregation load of deaths due to the MN locus is $tc^2/(1 + c)^2 + ct/(1 + c)^2 = ct/(1 + c) = .0436 \pm .0108$ per zygote, without allowing for additional deaths due to reproductive compensation. Assuming equal net fertilities of all mating types (complete compensation for deaths due to the MN locus) and $t = .0785$, a stable equilibrium is reached at $m = .5568$ with genotype frequencies $M = .3014$, $N = .1878$, $MN = .5108$. The excess of heterozygotes is measured by $\theta = 1.035$, in agreement with Table 1.

TABLE 4. POOLED SEGREGATION DATA AND ANALYSIS FOR $t = .093934$

Father	Mother	No of Families	Children						
			% MN	M	N	MN	Total	U_t	K_{tt}
Sources with families reported separately									
MN	M	279	52.05	374	•	406	780	−3.554	236.951
M	MN	310	50.00	456	•	456	912	−24.802	277.051
MN	N	213	49.68	•	319	315	634	−30.835	316.699
N	MN	197	54.88	•	254	309	563	14.082	281.232
MN	MN	518	55.29	338	317	810	1,465	Mvs.N 1.938	16.001
								M, Nvs.MN46.087	577.749
Sources with reciprocals pooled									
MN × M		356	50.97	428	•	445	873	−14.360	265.203
MN × N		267	48.36	•	331	310	641	−43.183	320.195
MN × MN		328	53.66	211	175	447	833	Mvs.N 4.835	9.430
								M, Nvs.MN9.139	328.508

	d. f.	χ^2	P
Deviation from $t = 0$	1	12.99	.0002
Between reciprocals	2	3.95	.2–.1
Among mating types, reciprocals pooled	2	9.75	.01–.001
Between MN × MN types	1	1.04	.5–.3
Among sources	40	58.15	.03–.02
Among families within sources	1687	1801.53	.03–.02

Despite the apparently good fit of this model, there is significant heterogeneity in t among mating types, the intercross giving more MN children, and the backcross to N fewer MN children, than expected. Heterogeneity between reciprocal MN × N crosses is almost significant ($\chi^2_1 = 3.24$, $P = .07$). The MN♂ × N♀ mating is incompatible for the M antigen and in Caucasian populations is incompatible also for the S antigen in about 37% of matings, in 69% of which the MN father is MSNs. Anti-S occurs as both a normal and immune antibody and is a rare cause of hemolytic disease. Anti-M has been found as a rare immune antibody and a very rare cause of hemolytic disease (Race and Sanger, 1954, p. 350). There is no basis for inferring the effect of these antibodies on early fetal death not recognized as hemolytic disease. The importance in fetal deaths of other components of the MNS locus remains to be determined.

The suggestion of maternal-fetal incompatibility is not supported by studies on the segregation ratio in families of different sizes. Table 5 shows the results for matings of MN♂ × N♀. If there were progressive maternal immunization by incompatible children, the proportion of MN children should decrease with sibship size. In fact, the regression of segregation frequency on family size is $-.0046 \pm .0086$. This may be contrasted with a highly significant decrease of incompatible children in matings of A♂ × O♀, the regression coefficient being $-.0274 \pm .0083$ on a com-

parable amount of data (Chung and Morton, in preparation). The failure of the regression for MN♂ × N♀ matings to approach significance casts considerable doubt on the hypothesis of M or S incompatibility, but neither have we been able to explain heterogeneity among mating types by positive technical errors or illegitimacy.

Formally the relative deficiency of MN children in backcrosses to N could be explained as the result of weak anti-M sera, an avoidable error which is less likely to occur than the use of weak anti-N sera (Wiener, 1943, p. 229). On this hypothesis the actual excess of heterozygotes must be greater than we have estimated it to be.

Even if the selection hypothesis is substantially correct, it may be considerably oversimplified. The segregation data suggest that MN excess is greatest for MN mothers and perhaps absent for M and N mothers. On this hypothesis, the segregation analysis for $t = .0939$ is as follows:

	d. f.	x^2	P
Deviation from $t = 0$	1	21.89	.00001
Deviation of reciprocals from expectation	2	3.95	.2–.1
Among mating types	2	5.33	.1–.05
Between MN × MN types	1	1.04	.5–.3

$t^* = .1157 \pm .0247$

The assumption of a maternal effect, being *ad hoc*, fits the data very well. The segregation load of deaths due to the MN locus would be about $ct/2(1 + c) = .0321 \pm .0069$ per zygote, without allowing for additional deaths due to reproductive compensation. Assuming equal net fertilities of all mating types and $t = .1157$, a stable equilibrium is reached at $m = .5853$ with genotype frequencies $M = .3340$, $N = .1634$, $MN = .5026$. The excess of heterozygotes at equilibrium is measured by $\theta = 1.035$.

THE TECHNICAL ERROR HYPOTHESIS

The above calculations are interesting only if the apparent excess of heterozygotes is not due to technical errors. Extramarital children cannot account for an MN excess in intercrosses because their distribution is $(mM + nN)(M/2 + N/2) = \frac{1}{2}$ MN + ½(M or N). However, positive typing errors (misclassifying M or N as MN) might simulate heterozygote excess.

Suppose that the probability of misclassification of type M as MN is v and type N as MN is w. The widely quoted assertion of Wiener (1943, p. 236), that technical errors are most likely to escape notice in apparent intercrosses, does not apply to positive errors (misclassifying M or N as MN) in either parents or children unless more than two alleles or generations are studied, since otherwise positive errors do not produce genetic exceptions in apparent intercrosses *or* backcrosses. There is abundant evidence that MN frequencies are the same in children and adults and that the M and N antigens are fully expressed at birth (table 4; Wiener, 1943, p. 230). For these reasons we must assume that the probabilities of positive errors in apparent intercrosses and backcrosses are the same in parents and children. Minimal estimates for v and w will be obtained by neglecting negative errors (misclassifying MN as M or N).

TABLE 5. SCORES FOR p = ½ BY SIBSHIP SIZE

s	*MN* ♂ × *N* ♀		*N* ♂ × *MN* ♀	
	Up	Kpp	Up	Kpp
1	−30	164	−2	188
2	20	528	28	392
3	2	516	44	504
4	8	448	36	448
5	−2	300	10	300
6	4	264	−20	240
7	6	28	—	112
8	12	128	8	32
9	−2	36	6	36
10	−8	80	—	—
11	−18	44	—	—
	b = −.00461 ± .00856		b = −.00558 ± .01167	

p = frequency of MN children

Let M, N, and H be the genotype frequencies of M, N, and MN, respectively, v and w in parents be denoted by v′ and w′, and p be the proportion of "MN" progeny expected. In M × "MN" matings, there are three possible genotypes for the "MN" parent.

possible genotypes	frequency	p
M × MN	H	(1 + v)/2
M × M	Mv′	?
M × N	Nw′	1

The value of p in M × M matings misclassified as M × "MN" depends on the heritability of such errors. If they arise from rare crossreactive alleles like M^c (Dunsford, Ikin, and Mourant, 1953) or from crossreactivity of anti-N with a strong *M* allele or a rare gene at another locus, p will approach ½ as the frequency of the gene approaches zero. On the other hand, if misclassification is due to weak residual activity for a common blood factor, p will approach v, the frequency of M misclassification in the general population. By adopting the first hypothesis, the estimate of v in children will be a minimum, and the probability of all "MN" children in a sibship of size s will be $y + (1 - y)p^s$, where p = (1 + v)/2, q = 1 − p, and y = Nw′/(H + Mv′ + Nw′). Maximum likelihood scores for p and y (Morton, 1959) may be transformed into scores for v, w, v′, and w′. Similarly, in N × "MN" matings the probability of all "MN" children is $y + (1 - y)p^s$, where p = (1 + w)/2, y = Mv′/(H + Mv′ + Nw′). In "MN" × "MN" matings with "MN" progeny excluded, the probability of all M children is $h + (1 - h)q^s$ and the probability of all N children is $y + (1 - y)p^s$, where the frequency of N children is p = (1 − w)/(2 − v − w), h = 2Mv′/(H + 2Mv′ + 2 Nw′), and y = 2Nw′/(H + 2Mv′ + 2Nw′). Including "MN" progeny and pooling M and N children, the frequency of "MN" is p = (2 + v + w)/4, with h = y = 0. Maximum likelihood scores for p, h, and y have been developed (Table 6), and a computing program for the IBM 650, written by R. Hedberg and Nancy Jones, is available through the Department of Medical Genetics, University of Wisconsin Medical School.

The results of this analysis are shown in Table 7. There is no significant difference

TABLE 6. MAXIMUM LIKELIHOOD SCORES FOR p, h, AND y

phenotype	G	g
frequency expected with segregation	q	p
observed in sibship of size s	s-r	r

$A = h + (1\text{-}h\text{-}y)q^s \quad B = y + (1\text{-}h\text{-}y)p^s \quad C = 1\text{-}h\text{-}y$

Case I: $r = 0$. $u_p = -Csq^{s-1}/A, \quad u_h = (1 - q^s)/A, \quad u_y = -q^s/A$

Case II: $r = s$. $u_p = Csp^{s-1}/B, \quad u_h = -p^s/B, \quad u_y = (1 - p^s)/B$

Case III: $0 < r < s$. $u_p = r/p - (s - r)/q, \quad u_h = -1/C, \quad u_y = -1/C$

$k_{pp} = (Csq^{s-1})^2/A + (Csp^{s-1})^2/B + Cs/pq - s^2Cq^{s-2} - s^2Cp^{s-2}$

$k_{hh} = (1 - q^s)^2/A + p^{2s}/B + (1 - p^s - q^s)/C$

$k_{yy} = q^{2s}/A + (1 - p^s)^2/B + (1 - p^s - q^s)/C$

$k_{ph} = -Csq^{s-1}(1 - q^s)/A - Csp^{2s-1}/B - sq^{s-1} + sp^{s-1}$

$k_{py} = Csq^{2s-1}/A + Csp^{s-1}(1 - p^s)/B - sq^{s-1} + sp^{s-1}$

$k_{hy} = -q^s(1 - q^s)/A - p^s(1 - p^s)/B + (1 - p^s - q^s)/C$

between v and w or v′ and w′, so that on the technical error hypothesis we must suppose that false positive reactions occur with about the same frequency for M and N antisera, despite the experience of Wiener (1943, p. 230) and others that errors are more frequent with anti-N. There is a gross discrepancy between v, w and v′,w′, indicating on the technical error hypothesis that false positive reactions are more common in children than adults. This conclusion is, however, contrary to all available evidence: equal MN frequencies in children and adults, presence of strong M and N antigens in early fetuses, titrations of infant and adult cells, and the inability of genetic tests to detect false positives in either generation of backcrosses and intercrosses. The contradiction is all the more striking, because in MN × MN matings the value of v′ and w′, determined from the frequency of nonsegregating families, is far smaller than the value of v and w, determined from the frequency of MN children ($\chi^2 = 11.5$, $P < .001$). We have seen that extramarital children cannot increase the frequency of MN children in this mating type, and false negative reactions could only reduce the apparent frequency of MN children. We must conclude either that false positive reactions are more common in children than adults, contrary to the available data, or that the apparent excess of MN children cannot be explained by technical errors.

Neglecting the above evidence, we might postulate that juvenile erythrocytes are for some reason more subject than adult cells to nonspecific agglutination by M and N antisera. This tendency should, however, be the same for backcrosses and intercrosses, yet the scores for v and w are significantly heterogeneous between the two mating types ($\chi^2_1 = 6.95$, $p < .01$). This heterogeneity, which can be accommodated by the selection theory, seems fatal to the technical error hypothesis.

There is another objection to an appreciable frequency of technical errors, even if we disregard evidence to the contrary and suppose that false positive reactions are more common in children than adults. For then the estimate of the frequency of such errors in children is .053 ± .014 on these data. Considering that some of these investigators must have used more than one antiserum of each type, the frequencies of discordant replicate tests with the same or different antisera and of exceptional "MN" children from N × N and M × M matings must be considerable. Yet Wiener (1943, p. 228) "has tested more than 1000 different blood specimens, using three

TABLE 7. ANALYSIS OF TECHNICAL ERROR HYPOTHESIS FOR v = w = v′ = w′ = .018891

Father	Mother	U_v	U_w	$U_{v'}$	$U_{w'}$	K_{vv}	K_{ww}	$K_{v'v'}$	$K_{w'w'}$	K_{vw}	$K_{vw'}$	$K_{wv'}$	$K_{v'w'}$
MN	M	11.248	—	—	3.649	758.295	—	—	448.576	—	247.434	—	—
M	MN	−23.378	—	—	−6.193	885.868	—	—	517.138	—	291.753	—	—
MN	N	—	−23.836	7.787	—	—	608.179	774.962	—	—	—	293.512	—
N	MN	—	38.290	12.004	—	—	539.128	643.142	—	—	—	273.801	—
MN	MN, M vs. N	—	—	−41.132	−15.673	—	—	1598.692	702.143	—	—	—	−282.672
	M, N vs. MN	86.326	86.326	—	—	574.705	574.705	—	—	574.705	—	—	—
Total		74.196	100.780	−21.341	−18.217	2218.868	1722.012	3016.796	1667.857	574.705	539.187	567.313	−282.672

	d.f.	χ^2	P
v vs. w	1	0.695	.5–.3
v′ vs. w′	1	0.020	.9–.8
(v, w) vs. (v′, w′)	1	5.962	.02–.01
Between reciprocals	2	1.788	.5–.3
Among mating types, reciprocals pooled	2	2.097	.5–.3
Between MN × MN types	1	11.499	.0007
Deviation from v = w = v′ = w′ = 0	1	10.798	.001

different anti-M fluids, without finding a single discrepancy among the reactions", and other investigators have reported similar results with smaller samples. In early studies on inheritance of the M-N types, only 13 contradictions to the theory of Landsteiner and Levine were found in 6718 children, all of them consistent with extramarital paternity, and not a single exception was found in 14601 mother-child pairs (Wiener, 1943, p. 233). In the whole of the material used for the present study, only a handful of exceptions were reported that were not exceptions also for other blood group systems, and all of these were consistent with extramarital paternity. The M-N system is considered reliable evidence in paternity suits and problems of identity (Andresen, 1947). Taylor and Prior (1939 a), the first investigators to draw attention to MN excess, could find no evidence of technical errors after careful examination of their data, and we have taken pains to exclude two studies where technical errors were suggested by a gross excess of MN parents. Of the six samples remaining with χ^2 greater than 4, only the small study of Hirszfeld and Kostuch (1938) gives an estimate of t significantly greater than the mean for other studies, and the deviation for all six studies pooled is not significant. On the evidence, we do not believe that technical errors can account for the observed excess of MN progeny.

ALTERNATIVE HYPOTHESES

The possible existence of an ambivalent $\overline{MN}$ allele with both M and N specificity has been alluded to. An individual carrying such an $\overline{MN}$ factor could also transmit either the *M* or *N* allele, but not both. Among MN parents there should be an excess of nonsegregating matings (only MN progeny from backcrosses, and only M but not N, or N but not M, progeny from intercrosses), but among segregating matings there should be no excess of the MN type. In terms of the above model, we would expect $v = w = 0$ (corresponding to no excess among segregating families), but both v' and w' to be greater than zero (corresponding to an excess of nonsegregating matings). On the contrary, v' and w' are significantly *less* than v and w ($P < .01$). The data clearly cannot be explained by ambivalent alleles.

The recent demonstration of trisomy as the cause of mongolism (Lejeune *et al.* 1959) suggests that non-disjunction might explain the excess of MN progeny. Nondisjunction for the pair of chromosomes that carry the MN locus would give rise to normal, disomic, and nullisomic gametes from MN parents in the frequencies

$$(1 - x)/2 \quad M \qquad (1 - x)/2 \quad N \qquad x/2 \quad MN \qquad x/2 \; —,$$

where x is the frequency of nondisjunction. If all the zygotes from these gametes were functional, there would be no excess of heterozygotes. From trisomic MN parents, between $\frac{2}{3}$ and $\frac{5}{6}$ of the progeny would be of the MN phenotype in unions with gametes carrying the minor allele (as N in MMN) and between $\frac{1}{3}$ and $\frac{1}{2}$ in unions with the major allele (as M in MMN) depending on centromere distance and transmission of disomic gametes. The frequency of normal aneuploid individuals would have to be at least several per cent to account for MN excess.

There are several serious objections to the above hypothesis. With the best cytological techniques, the normal karyotype of man has invariably been found to be

euploid in over 100 individuals studied (Chu and Giles, 1959). The hypothesis of non-disjunction requires a considerable frequency of aneuploidy for this one chromosome, and presumably similar frequencies for other chromosomes. Polysomy per se would give no stable MN polymorphism. Finally, there is no evidence of an effect of age of mother on the frequency of MN children, as tested by the analysis of sibship size (Table 5). The marked increase of mongolism with maternal age suggests that non-disjunction or survival of aneuploids may be related to maternal age in man. However, the generality of this observation cannot be established from mongolism alone, and we must await studies on other trisomics before attempting to evaluate the association between maternal age and aneuploidy. Analyses of somatic loss of ring-X chromosomes in Drosophila (Brown and Hannah, 1952) are presumably irrelevant, because of the peculiar configuration and instability of such chromosomes. At this stage in human genetics, the best argument against trisomy as a cause of MN excesses would seem to be the improbability that any appreciable frequency of normal aneuploidy would have escaped the attention of cytologists.

At the present time it is not known whether the MN locus affects the antigenic or other physiological properties of sperm or whether, if so, genetic segregation is reflected by physiological differentiation of the two classes of sperm from an MN male. Without anticipating the results of such studies, we may consider whether preferential fertilization by heterologous sperm could account for the observed MN excess.

This hypothesis is certainly consistent with failure to find evidence of sterility or abortion due to the MN locus, and the only critical data come from genetic segregation. In all matings of MN males, the probability of an MN child would be $1/(1 + f)$, where f is the relative fitness of sperm of the same M-N type as the egg. If the father is not of type MN, but the mother is, the probability of an MN child would be $\frac{1}{2}$. When reciprocal crosses are pooled, the probability of an MN child would be $(3 + f)/4(1 + f)$.

When the available data are tested against this hypothesis, the high frequency of MN children from N$\times$ MN matings makes heterogeneity between reciprocal crosses significant ($\chi^2_2 = 8.10$, $p < .02$). The overall fit is no better than for the hypotheses of simple postzygotic selection and technical errors. Considering also the delicate complementarity of egg and sperm that must be postulated, we may tentatively reject preferential fertilization by heterologous sperm as an explanation of MN excess.

A fortiori, the same tests which exclude the technical error hypothesis also rule out somatic mutation as the cause of MN excess, since such mosaicism would be cumulative and hence greater in parents than children, and would give rise to genetic exceptions in M $\times$ M and N $\times$ N matings.

Formally, the fact that MN excess is greatest for MN mothers, and perhaps limited to them, could be explained as a transmitted maternal effect. Since erythrocytes are continually being produced and destroyed, the only conceivable mechanism for such an effect would require tolerance by the progeny of maternal erythropoietic tissues implanted during fetal life, in which case the child would presumably tolerate maternal homografts. Furthermore, the absence of an excess of nonsegregating parents requires the ancillary hypothesis that such tissues, though tolerated during child-

hood, are eliminated before reproductive maturity. There is no basis for either of these suppositions, which are also inconsistent with the observed equality of MN frequencies among parents and children.

Meiotic drive (Sandler and Novitski, 1957) would produce no stable equilibrium or excess of heterozygotes unless the distortion were equal in magnitude and opposite in direction in the two sexes. In that case, the selection pressure toward equilibrium would be extremely small, and the excess of heterozygotes would be considerably greater in some backcrosses than in intercrosses.

We tentatively conclude that MN excess, if it cannot be due to technical errors, must reflect heterozygote advantage.

DISCUSSION

There are two hypotheses for heterozygote advantage: (1) independent action of alleles, the product of one being adaptive at some stage of development and the product of the second at another stage; (2) allelic interaction to produce effects that are quantitatively or qualitatively different from the sum of the homozygous effects. The latter model includes the hybrid substances which have been found in animals but never looked for in man (Irwin, 1951). Both hypotheses require that the MN locus have other and more important physiological effects than the production of erythrocyte antigens.

Whatever the physiological basis for MN advantage, there should be an excess of heterozygotes in random mating populations. However, any subdivision of the population into isolates would tend to increase homozygosis. This, in conjunction with the loss of information when gene frequencies must be estimated from the sample, makes conventional anthropological studies much less efficient than family investigations as a test of heterozygote excess.

If the MN type is advantageous, we might expect that duplications or ambivalent alleles, with both M and N specificity, would increase in the population, whereas they are extremely rare (Dunsford, Ikin, and Mourant, 1953). However, such alleles are not identical with the MN phenotype, and need not have the same fitness. Gene duplications often have unfavorable side effects, generally ascribed to gene dosage, and it is conceivable that genes may sometimes interact unfavorably in the cis position.

Although it is not difficult to defend the hypothesis of MN advantage on theoretical grounds, we must beware of the fallacy which Fontenelle signalized in his *Histoire des Oracles*: "Je ne suis pas si convaincu de notre ignorance par les choses qui sont, et dont la raison nous est inconnue, que par celles qui ne sont point et dont nous trouvons la raison. Cela veut dire que non seulement nous n'avons pas les principes qui mènent au vrai, mais que nous en avons d'autres qui s'accommodent très bien avec le faux." The important question at this time is not how MN advantage may be explained, but whether it does, in fact, exist. The evidence is essentially indirect, in that the hypothesis of technical errors does not seem to be tenable. In the absence of any suggestion of differential viability or fetal death, the segregation analysis assumes critical importance. No direct test of errors in these published studies is

possible, and it is therefore necessary to consider the kind and amount of data required to refute or confirm the selection hypothesis decisively.

The material should come, if possible, from a population not practicing birth control, such as the Hutterites, where early fetal death might be reflected by the interval between children. Children of MN mothers provide the critical material, free from any need to exclude extramarital paternity. The S,s and other components of the MN locus should certainly be studied, since they may influence MN selection. To control technical errors, several independently prepared anti-M and anti-N reagents, constant throughout the study, should be used with titration and quantitative scoring, the tests being repeated on a random sample of cells. In this way the frequency of misclassification would be both minimized and accurately known. The sample size should be of the order of thousands. Until such data are collected, the hypothesis of MN advantage should neither be accepted uncritically, nor rejected in favor of the hypothesis of technical errors, which on the evidence appears to be untenable.

SUMMARY

In published European and American family studies there is no significant association between MN type and fertility or abortion. The excess of MN children in back- and intercrosses is highly significant and consistent with heterozygote advantage, apparently limited to children of MN mothers. Selection intensities estimated from the data would produce the observed genotype frequencies at equilibrium. The data are not consistent with the hypotheses of illegitimacy, false positive reactions, ambivalent alleles, non-disjunction, preferential fertilization, somatic mutation, or meiotic drive to explain MN excess. Requirements for a direct demonstration or refutation of heterozygote advantage are discussed.

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Quantitative Genetics of Palmar Dermatoglyphics

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FOR MANY BIOLOGICAL INVESTIGATIONS, the use of quantitative traits is preferable to the use of qualitative ones, whenever the former prove to be sufficiently representative of the phenomenon or process analyzed.

In the particular case of the palmar dermatoglyphics, it has been shown that the general aspect of the palmar configurations is determined by the courses of the main lines A, B, C, and D (Fig. 1), these courses being more or less oblique. For analyzing the degree of obliquity, it is convenient to assign a numerical expression to the trait. For this purpose the different segments around the periphery of the palm are numbered, so that the termination of each main line may be recorded. Thus in Fig. 1 main line D terminates at 11 and is scored as 11.

As a quantitative expression of the degree of obliquity, we use the value resulting from the addition of the main-line indices for both hands (Cummins 1942; Cummins and Midlo 1943), which has the advantage of expressing the obliquity of the lines of the palm by means of a single quantity. This index makes use of lines A and D alone. It is not a proportion; it is the sum of the values attached to lines A and D. Values are assigned to the positions used in the scheme of formulating main lines (Fig. 1). For line A the original numerical symbols are adopted as values, with the single exception that value 6 is assigned to position 5″, and for line D, values 1 through 8 are substituted for positions 6 through 13, respectively. Thus the progressively higher values of both lines indicate an equivalent approach to the transverse direction. For more details see the papers by Cummins.

Concerning the distribution and degree of heritability of the palmar main lines, it should be remembered that the differentiation of the dermatoglyphics occurs during the third and fourth months of intrauterine life and that the lines remain constant after birth. Therefore, except in size, the dermal configurations do not vary with age and are practically independent of environmental effect. On the other hand, it is obvious that there is no assortative mating, such as happens, for example, with skeletal proportions and pigmentation.

MATERIAL

The material analyzed consists of a total of 647 Spaniards (295 males and 352 females), including unrelated persons and family data. For the study of main-line index distribution, a series of 200 males is used and another one of 200 females, both comprising unrelated persons only. To analyze the degree of heritability, we have a series of 286 sibs falling into 113 sibships.

Received September 19, 1958.

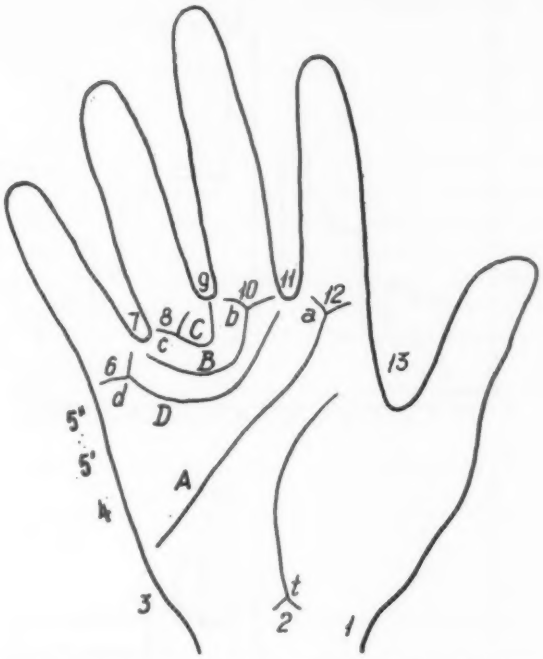

FIG. 1.—Main lines of the palm (*a*, *b*, *c* and *d* are the digital triradii, which proximal radiant are the main lines A, B, C and D respectively; *t* is the axial triradius).

More than 85 per cent of the individuals come from the northeastern provinces of Spain, and since the predominant racial element there is the Mediterranean, we believe that this element is the most frequent one in our series.

MAIN-LINE INDEX DISTRIBUTION

From an analysis of the series of unrelated individuals (200 males and 200 females), the following means are obtained: male = 16.09 ± 0.27; female = 16.45 ± 0.28. The difference between the two means is not significant (t = 0.93 for 398 degrees of freedom). According to published data by different authors, the sex differences in palmar main lines are, as a rule, slight and are probably due to chance.

In the histograms of Fig. 2 it is seen that the range of variation lies between 7 and 24. For each sex the deviations from normality (skewness and kurtosis) were calculated by Fisher's method (Fisher 1954). The values g_1 (skewness) and g_2 (kurtosis) obtained for each sex are

$$\text{males: } g_1 = -0.132 \pm 0.172;\ g_2 = -0.696 \pm 0.342$$
$$\text{females: } g_1 = -0.286 \pm 0.172;\ g_2 = -0.799 \pm 0.342$$

Both distributions are therefore negatively skew, but in both cases, the values of g_1 are not significant. Both distributions are significantly platikurtic; for males being

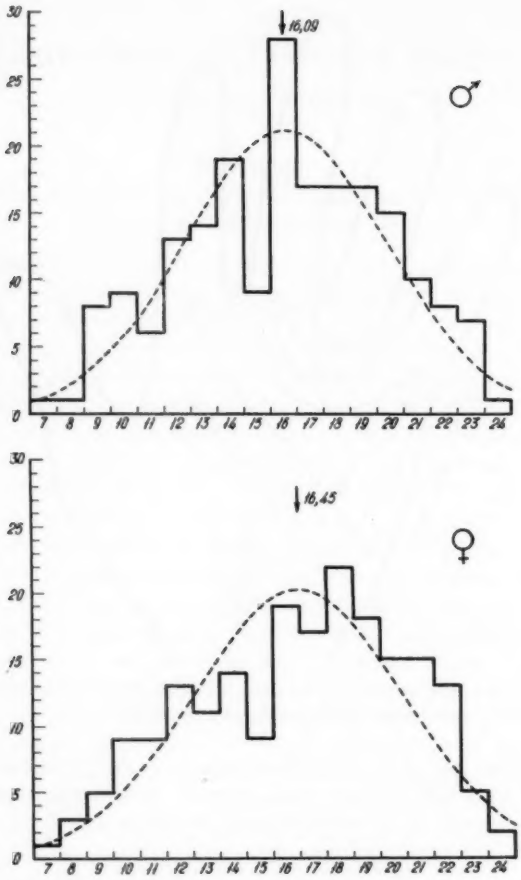

FIG. 2.—Main-line index distribution in Spaniards (200 ♂ and 200 ♀) and the calculated norma curve with the same mean and standard deviation.

somewhat below the 5 per cent level ($t = 2.04$) and for females practically coinciding with that of 2 per cent ($t = 2.34$).

To determine the deviations from normality, larger series are necessary. Since no significant sex differences appear between the parameters of the two series, these may be combined into one series, which shows intermediate values (mean = 16.27 $\pm$ 0.19; $s = 3.87 \pm 0.14$; $g_1 = -0.207 \pm 0.122$; $g_2 = -0.767 \pm 0.243$). The negative skewness is still not significant, while the platykurtosis is now significant at the 1 per cent level. Use of the remaining sibship data increases the statistical information, though not equivalent to their total number because of their relationship. These data added to the unrelated ones, make a total of 647 (295 males and 352

females). The values obtained for this series agree with the former ones (mean = 16.38; s = 3.75; $g_1 = -0.181$; $g_2 = -0.742$).

SIB PAIR CORRELATION

In the series of 286 individuals distributed among 113 sibships, the sibships of two members account for two thirds of the total number.

To determine the degree of likeness between sibs, we have calculated the intraclass correlation. First, a "symmetrical table" of correlation has been worked out. This table joins 568 points, in other words, twice the 284 pairs obtained from all the pairs of observations within each sibship. The 284 sib pairs were distributed as follows: 77 from sibships with two members; 69 from sibships with three; 48 from sibships with four; 30 from sibships with five; 15 from sibships with six; and 45 from one sibship with ten members. Calculating the correlation by means of the ordinary product-moment, a coefficient of 0.45 resulted.

More adequate is the calculation of the intraclass correlation based on the analysis of variance. This analysis is summarized as follows:

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Between classes	2552.33	112	22.79
Within classes	1183.50	173	6.84
Total	3735.83	285	

The intraclass correlation was obtained from the following formula:

$$\rho = V_M/(V_M + V_W)$$

where V_W is the variance within classes, which may be estimated from the mean square within classes, and V_M the variance between classes obtained from the formula:

$$V_M = N(k-1)\,[N^2 - \Sigma n_a^2]^{-1}\,(msq_B - msq_w)$$

where N is the total number of sibs, k that of the sibships, n_a that of the sibs in each sibship, while msq_B and msq_w are the mean squares between and within classes, respectively. We obtained $V_M = 6.31$, that with $V_W = 6.84$ give a sib pair correlation equal to 0.48. This value remains somewhat above that calculated by the symmetrical table and approaches, therefore, even more closely the theoretical value of 0.50, which is the maximum possible correlation between sibs, when the genetic determination is due to polygenes with additive effect.

These results confirm those obtained in a previous paper (Pons 1954) based on many fewer data. (The present paper includes the previous data.) In the previous paper, the parent-child correlation and the parent-parent correlation were determined. The former was r = 0.52, while the latter was negligible ($r = 0.001 \pm 0.14$) which seems to eliminate the possibility of assortative mating for this trait and, consequently, permits the interpretation that the correlation obtained between sibs is its full value.

Furthermore, in the paper referred to above, a high correlation was found for a short series of uniovular twins. The coefficient obtained ($r = 0.95$) is consistent with that obtained for the sample of sibs ($r = 0.48$).

SUMMARY

The transverseness of palmar main lines expressed by the sum of the Cummins main-line index for each palm can be analyzed as a quantitative trait, whose frequency distributions show a small negative, though not significant skewness, and a significant platykurtosis in the series here studied. The intraclass correlation between sibs and the parent-child correlation are near 0.50. No assortative mating is present, for there is no correlation between parents. Thus the results obtained do not disagree with the hypothesis of a polymerie with genes of additive effect.

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An Investigation Into The Biochemical Genetics of β-Aminoisobutyric Aciduria*

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INTRODUCTION

β-AMINOISOBUTYRIC ACID (BAIB) is a non-protein amino acid found in human urine in widely varying concentrations from one person to another. Several studies have demonstrated that this variation is largely under genetic control, with most of the variability being due to differences at a single locus (Harris 1953; Gartler, Firschein and Kraus 1957). The present report is concerned with the question of the mode of action by which the genetic differences lead to the observed variability in urinary BAIB excretion rates.

Crumpler, Dent, Harris, and Westall (1951), in their original paper describing β-aminoisobutyric aciduria, suggested that the underlying mechanism in this condition was a genetically controlled renal tubular defect. The supporting evidence for this hypothesis was the fact that they did not find gross differences between the blood levels of BAIB in individuals with β-aminoisobutyric aciduria (high excretors) and individuals excreting only small amounts of BAIB (low excretors). They argued that if a block in the intermediary metabolism of BAIB, rather than a renal defect, were responsible for this amino-aciduria, the plasma level of BAIB would be markedly elevated in high excretors.

For substances which are normally reabsorbed from the glomerular filtrate with a high degree of efficiency (e.g., protein occurring amino acids), this argument is valid. However, when a substance is normally reabsorbed with a low efficiency, a block in intermediary metabolism will not lead to its accumulation in the blood (e.g. alcaptonuria), and in such instances, comparative blood levels for the substance might not be critical. Nothing is known about the normal absorption rate for BAIB. Furthermore, though the immediate cause of an aminoaciduria may be renal, the primary genetic lesion may be one of intermediary metabolism which indirectly brings about renal damage and aminoaciduria (e.g., galactosemia and Wilson's disease).

In view of these uncertainties, it appears that the question of the underlying mechanism in β-aminoisobutyric aciduria is unanswered, and the following studies were undertaken to investigate this problem more fully.

MATERIALS AND METHODS

BAIB and related substances (thymine, DHT and BUIB)[1] were administered to normal high and low excretor subjects (Table 1). BAIB, thymine, and DHT were

Received February 5, 1959.

* Supported by grants from the National Science Foundation (G-4464) and the Public Health Service (H-3091).

[1] DHT = dihydrothymine. BUIB = β-ureidoisobutyric acid.

TABLE 1. AGE, SEX, WEIGHT, AND FASTING BAIB EXCRETION VALUES FOR THE NORMAL SUBJECTS USED IN THESE EXPERIMENTS

Subject	Age	Sex	Weight	Fasting Urinary BAIB Excretion Mg. BAIB in 6 hours*
Low Excretors				
SG	34	M	69 Kg.	5.3
LL	22	M	60	2.5
BV	27	M	67	3.3
PC	21	F	52	3.1
JS	20	F	63	6.0
BW	40	F	56	3.4
High Excretors				
HW	20	M	60	45.7
JA	24	M	65	44.1
ML	32	F	45	34.3
RG	21	F	51	55.4

* Based on at least two determinations taken on different days.

obtained from the California Foundation for Biochemical Research. BUIB was prepared in the laboratory according to the method of Fink, McGaughey, Cline and Fink (1956). At appropriate times, blood and urine specimens were taken and analyzed for BAIB and, in some cases, for thymine, DHT, and BUIB. The data thus obtained permitted (1) analysis of the normal renal reabsorptive values for BAIB, (2) comparison of renal function with regard to BAIB in high and low excretors, and (3) comparison of the metabolic pathway involving BAIB in high and low excretors.

Urine specimens were either analyzed the day of collection or stored in a frozen state until analyzed. Serum was treated with four volumes of ethanol, the precipitate removed by centrifugation, and the ethanolic extract used directly for analysis.

BAIB determinations were carried out by high voltage electrophoresis on paper (Gartler 1959). DHT and BUIB determinations were made by the methods of Fink, McGaughey, Cline and Fink (1956). Thymine was detected by examining paper chromatograms under ultra violet light.

RESULTS

Renal Excretion of BAIB in High and Low Excretors

It has not been possible to detect BAIB in the fasting serum of any of the subjects investigated, even though the methods used were sensitive to less than 0.1 mg. per cent of serum BAIB. The average BAIB urinary excretion rates reported in Table 1 serve to class BAIB as a low threshold substance or one that is normally reabsorbed with a low degree of efficiency. At such low blood concentrations (less than 0.1 mg. per cent) the average minimal renal clearance for the high excretors approaches the glomerular filtration rate, while for the low excretors, the average minimal renal clearance would be many-fold greater than that for comparable substances, such as other amino acids.

Feeding experiments were undertaken to increase the serum BAIB to measurable

TABLE 2. RENAL CLEARANCES OF BAIB IN LOW AND HIGH EXCRETORS AFTER THYMINE ADMINISTRATION

Subjects	Mg. Thymine Administered	Mg. BAIB Ml. Serum	Mg. BAIB Excreted/ minute	Renal* Clearance
Low Excretors				
PC	500	0.0025	0.39	156.0
PC	1500	0.0058	1.47	253.4
LL	500	0.0050	0.80	160.0
SG	1500	0.0060	1.43	238.3
JS	1500	0.0045	0.94	208.9
BV	500	0.0020	0.35	175.0
High Excretors				
ML	500	0.0031	0.50	161.3
ML	1500	0.0060	1.47	245.0
RG	500	0.0080	0.78	97.5
RG	1500	0.0100	1.45	145.0
HW	500	0.0045	0.60	133.3

* Ml. serum cleared/minute.

levels. Thymine, a highly effective precursor of BAIB in man (Awapara and Shullenberger (1957), Gartler (1959)) was orally administered. The BAIB excreted after thymine administration is the L form, which is excreted in β-aminoisobutyric aciduria (Gartler, unpublished data). Blood samples were obtained two hours after thymine administration and urine specimens were collected fifteen minutes before and fifteen minutes after the drawing of blood samples. Table 2 lists the results of these experiments. As can be seen, there is no evidence of tubular reabsorption of BAIB at these blood levels (0.2 mg. per cent–1.0 mg. per cent). The renal clearances are all very high, and with one exception, exceed the normal glomerular filtration rate. Of particular interest are those instances where increasing dosages of thymine were administered to the same individuals (P. C., M. L., and R. G.). In all cases, the renal clearances were increased to such high levels as to indicate the possibility of active tubular excretion of BAIB.

METABOLISM AND EXCRETION OF ADMINISTERED DL-BAIB IN HIGH AND LOW EXCRETORS

In view of the results of the preceding experiments, I decided to investigate the effects of the administration of BAIB, though the only form of this substance available was the racemic mixture. Table 3 presents the results of the experiments involving the oral administration of DL-BAIB to high and low excretors of BAIB. The low excretors retained almost all of the administered BAIB, whereas the high excretors excreted approximately 50 per cent of the administered material.

To circumvent the possible complications of variable intestinal absorption, BAIB was administered intravenously at two dosages to one low and one high excretor subject. Urine specimens were collected hourly, and it was found that normal BAIB excretion rates were resumed approximately one hour after BAIB injection. The results of these experiments are given in Table 4. They support the conclusions of the experiments involving the oral administration of BAIB. Blood specimens drawn

TABLE 3. URINARY EXCRETION OF BAIB AFTER THE ORAL ADMINISTRATION OF 50 MG. DL-BAIB

	Mg. BAIB excreted in 6 hours (Corrected for fasting BAIB level)
Low Excretors	
SG	2.4
BW	8.3
BV	3.5
High Excretors	
ML	37.2
JA	24.4
HW	23.1

TABLE 4. URINARY EXCRETION OF BAIB FOLLOWING THE INTRA-VENOUS INJECTION OF DL-BAIB

Subject	Mg. DL-BAIB Injected	Mg. Injected BAIB* Excreted in the Urine
Low Excretor (S. G.)	10	0.7
	20	2.9
High Excretor (H. W.)	10	7.2
	20	9.0

* Over 90 per cent of the recovered BAIB was excreted in the first hour following BAIB injection.

from the low excretor in the 20 mg. experiment at three minutes and 90 minutes after BAIB injection gave values of 0.47 and less than 0.10 mg. per cent, respectively. The 2.9 mg. excreted in the urine accounts for only a small fraction of the reduction in serum BAIB. It seems clear, therefore, that the major portion of BAIB in the low excretor is cleared by a different mechanism from that used by the high excretor. The fact that a considerable amount of the injected BAIB was retained by the high excretor in the 20 mg. experiment indicates that the high excretor may also use more than one mechanism for BAIB clearance.

An interesting sideline of the DL-BAIB experiment is that the low excretors apparently do not discriminate between the D and L forms of BAIB. For most amino acids, this would be unusual, but in the case of BAIB, which is not a structural substance, the absence of stereoisomeric specificity may not be critical.

DISCUSSION

There are at least three possible mechanisms of gene action which can lead to a genetically determined aminoaciduria; (1) renal defect, (2) block in precursor metabolism, and (3) differential utilization.

The data presented in the first section of this paper give no evidence for any significant difference in renal function between low and high excretors. In fact, there is no evidence for tubular reabsorption of BAIB at the serum levels studied in either high or low excretors, which argues strongly against the renal hypothesis of β-aminoisobutyric aciduria.

The possibility of a block in precursor metabolism (thymine $\rightleftarrows$ DHT $\rightleftarrows$ BUIB $\rightarrow$ BAIB) was investigated in a previous study (Gartler 1959), and was excluded as the underlying factor in β-aminoisobutyric aciduria.

In contrast to the preceding negative evidence, the experiments involving BAIB

administration have demonstrated a striking difference between low and high excretors in BAIB utilization. Whereas low excretors retain nearly all of the administered load, high excretors eliminate 50 per cent or more of it. Furthermore, the disappearance of the injected BAIB from the blood of the low excretor would make it seem most likely that we are dealing with differential metabolism of BAIB. Just what the difference is between low and high excretors in their utilization of BAIB is not at all clear. Conversion of BAIB to BUIB by low excretors could explain this difference, but there is no evidence for significant *in vivo* reversibility of the BUIB → BAIB step. Fink, McGaughey, Cline, and Fink (1956) found no BUIB after BAIB administration in the rat, nor was any BUIB detectable in either blood or urine specimens after relatively large dosages of BAIB (250 mg.) in these experiments. Kupiecki and Coon (1957) have shown that, in the pig, BAIB is involved in the following reaction:

β-aminoisobutyrate + α-ketoglutarate ⇄
glutamate + methylmalonate semialdehyde.

This is a possible mechanism for the differential metabolism of BAIB, but thus far we have not been able to demonstrate this reaction in human tissues. Other possibilities exist (e.g., BAIB → isobutyric acid) but as yet, no definitive evidence has been obtained on this point.

SUMMARY AND CONCLUSIONS

An investigation into the biochemical genetics of β-aminoisobutyric aciduria has indicated that the most likely explanation of the difference between high and low excretors is differential metabolism of BAIB. Studies have shown that no significant difference in renal function exists between low and high excretors, nor is there any metabolic block in the pathway leading to BAIB formation. However, there is a marked difference between low and high excretors in their utilization of administered BAIB. The exact nature of this last step is not known, but various possibilities are discussed.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the volunteers who participated in these experiments and to Dr. Arno G. Motulsky for his thorough review of the manuscript.

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Heritability in Dental Caries, Certain Oral Microflora and Salivary Components[1]

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INTRODUCTION

STUDIES OF THE ETIOLOGY of dental caries have suggested that both environmental and genetic factors may be concerned in the development of dental decay. Several major genes have been identified which condition defective formation of enamel or dentin, and rampant caries usually appears in such teeth (Witkop, 1958). However, these genes are rare and probably account for only a small proportion of all individuals with carious teeth. The role of genetic factors in the commonplace variety of dental caries remains uncertain. The few twin studies of ordinary dental caries have provided equivocal conclusions (Böök and Grahnen, 1953). The present twin study was begun to learn whether the use of measures more refined than those previously employed would reveal significant heritability in the common type of tooth decay.

The voluminous literature regarding etiological agents in dental caries includes many reports of exogenous and endogenous factors which may influence the frequency of dental decay. If, as seemed likely, genetic factors do participate in regulating susceptibility to decay, then study of the heritability of individual factors possibly related to tooth decay might permit partial separation of this complex. Hence, numbers of certain oral microflora and the rate flow, pH, and amylase activity of saliva were determined in a series of twins in whom the frequency of dental caries was also being measured. This paper reports on the heritability estimates calculated for dental caries and for the salivary characteristics measured.

MATERIALS AND METHODS

Twins. The 38 like-sexed twin pairs (19 monozygotic, 19 dizygotic) for the present study were with four exceptions students attending either Michigan State University (16 pairs) or junior or senior high schools in Lansing, Michigan (18 pairs). Ages ranged from 14 to 38 years with a median of 19 years. Only two pairs were over 24 years of age. All except 5 pairs were living and eating together either at University dormitories or at home. The monozygotic series included 9 male and 10 female pairs and the dizygotic twins, 5 male and 14 female pairs.

Zygosity was determined from serologic tests using 15 antisera for groups ABO

Received January 14, 1959.

[1] Aided by grants from the National Institutes of Health (D-791), the All-University Research Fund of Michigan State University, and the Dental Division of the Michigan State Health Department.

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(2), MN (2), Rh (5), Lutheran, Kell, Kidd, Duffy, P, and S. Any pair exhibiting differences in any of these tests was classified as dizygotic. In addition, any pair which showed a marked dissimilarity in eye or hair color was considered dizygotic. Anthropometric measurements (35) made by Dr. Philip J. Clark on the same twins for another purpose corroborated zygosities determined by non-serologic methods.

Dental Caries. The frequency of dental caries was determined by clinical examination employing explorer and mirror coupled with an interpretation of full mouth X-rays. To minimize exposure to radiation of both operator and subjects, X-rays were taken using one second exposures with Kodak Ultra-speed film. A lead diaphragm and aluminum filters were employed to further reduce exposure time and areas exposed. By employing these procedures, exposure time was reduced by 78 percent, the facial dose was reduced by 96 percent, and gonadal dose was reduced by 92 percent (Richards, 1958). Coded summaries of the clinical and X-ray findings were recorded on forms kindly provided by the Dental Division of the Michigan State Health Department.

Analyses of dental caries were based on the ratio of the number of decayed and filled tooth surfaces to the number of surfaces available to decay times 100. We have used the term "caries experience ratio" (CER) proposed by Horowitz, Osborne, and DeGeorge (1958) to describe this relationship. All teeth were considered to have 5 surfaces. Unerupted teeth, congenitally missing teeth, and teeth removed because of trauma or for orthodontic advantage were excluded from both numerator and denominator. Teeth extracted because of advanced caries and teeth bearing restorative full crowns were tabulated as having had 3 decayed surfaces prior to removal or restoration (Bodecker, 1939). In all tabulations, a single surface decay was considered equivalent to a single surface filling with the exception that an active caries on the mesial or distal surface of any posterior tooth was scored as a two-surface lesion because, in filling such decays, it is generally necessary to approach from the occlusal surface. This adjustment was not necessary if the tooth had an active caries or a filling on its occlusal surface because no provision was made for multiple carious foci on a single surface. All clinical examinations and interpretations of x-rays were made by the same operator (J. E. L.). The dental examinations of a given pair of twins did not always occur on the same day, but were usually given within the same week.

Microflora. Paraffin-stimulated saliva was collected from both members of each twin pair during the same morning. Subjects were instructed to deposit 15 to 20 ml. of saliva into sterile vials on first rising before brushing teeth or consuming any food or liquid. Saliva specimens were refrigerated immediately after collection. They were plated as soon as possible, usually on the afternoon of the day they were collected. The interval between collection and plating of specimens from a given twin pair was always the same for both members of the pair. Ten fold dilutions were made up in 0.05% yeast extract water. One-tenth ml. quantities of the appropriate dilution (that dilution yielding a readily countable plate) were pour plated using the following media: tryptone glucose extract (TGE) containing 4 percent skim milk for the growth and subsequent count of miscellaneous bacteria; Rogosa SL agar, modified slightly by substituting for half of the glucose equal amounts of sucrose and arabinose for

growth of lactobacilli; crystal violet azide blood agar[3] (CVA) for culture of streptococci. All plates were incubated aerobically at 37°C. The TGE and CVA plates were incubated for 3 days and the Rogosa SL agar plates were incubated for 4 days.

Rate of flow, pH, and amylase activity of saliva. The work of many prior investigators (see review by Cox, 1952) and preliminary work of the present study indicated that the rate of flow, pH, and amylase activity of saliva are influenced by a large number of environmental and physiological factors. Intrapair diurnal variation and the influence of mealtime were controlled to some extent by invariably collecting specimens simultaneously from both members of each twin pair. However, there was considerable interpair variability in time of day of saliva collections because of variations in the time when both members of each pair were free of other commitments. With the exception of 3 monozygotic pairs, both members of each pair had eaten at approximately the same time prior to collection. As mentioned above, all but five pairs were living and eating together either at home or at University dormitories.

In addition to diurnal physiological variations, whole saliva is heterogeneous because of anatomical, histological, and physiological differences between the three paired glands which produce it. This heterogeneity could best be controlled by collecting the secretions of each pair separately. However, the methods presently available for separating the secretions of the submaxillary from those of the sublingual glands involve either cannulation of Wharton's ducts or preparation of individual casts of the mouth. Since neither of these procedures seemed practicable, a mixture of sublingual and submaxillary secretions were collected from the floor of the mouth. At the same time, parotid secretions were collected through the use of plastic discs designed by Curby (1953) for this purpose.

Following an explanation of the technique, subjects were instructed to clear their mouths of saliva. Curby discs (held in place by vacuum) were then centered over Stenson's ducts. Subjects were reminded not to swallow during the period of collection. Within a minute or two saliva appeared in collecting tubules if the subjects were copious salivators. From time to time a tubule was passed over the floor of the mouth to collect accumulated sublingual-submaxillary (S & S) saliva. Salivas were collected in graduated centrifuge tubes to measure volume. The duration of collection was determined by the time needed to collect sufficient parotid saliva (about 0.5 cc.) for test procedures and varied from 7 to 52 minutes. Fastening of Curby discs and the passing of a tubule over the floor of the mouth provided stimuli for salivation.

The collection of saliva was not without problems. In a few subjects Stenson's ducts were located too high in the buccal pouch to permit easy attachment of the Curby devices. It was of incidental interest that a marked similarity in the position of Stenson's ducts and in the anatomy of the papillae surrounding them was noted in monozygotic twins but considerable intrapair variability was observed in the same trait among dizygotic twins. The following standard was adopted for checking on the attachment of the Curby devices: if saliva had not appeared in collecting tubules

[3] This medium comprises: brain heart infusion agar (Difco), 52 gm; agar, 5 gm; crystal violet, 1 ml. of a 0.2 per cent aqueous solution; sodium azide, 100 mg; and distilled water, q. s. 1 liter. Defibrinated sheep's blood (4 ml. per 150 ml. media) is added to cool, melted agar prior to use.

within 5 minutes, it was presumed that the devices were improperly placed and they were removed and replaced. More often than not, the devices had been properly placed, and failure to observe saliva was due to the subject's slow rate of flow. Some individuals swallowed S & S saliva in spite of reminders.

Rate of flow was estimated from recorded volume and duration of collection. The pH of specimens was measured with a potentiometer immediately following collection. Amylase activity was measured in duplicate using a modification of the method of Myers, Free, and Rosinski (1944) for determination of serum amylase. Amylase determinations were usually completed within 12 hours following collection and specimens were refrigerated at 4°C until tested.

THE DATA

Caries. All mean intrapair variances were computed from the formula: $V = \Sigma d^2/2n$, in which V is the variance, d is the intrapair difference, and n is the number of pairs. The calculated ratio between the variance in dizygotic twins and the variance in monozygotic twins (F test) was considered to support the hypothesis that genetic factors significantly influence the trait being studied if the probability (P) of getting a ratio as large or larger by chance was less than .05. A comparison of mean intrapair variances based on the CER for all teeth is presented in Table 1. Corresponding data for the separate quadrants of the mouth shown by many investigators to have different caries incidence rates are also given. Anterior teeth include the centrals, laterals, and cuspids, and posterior teeth include the first and second bicuspids, and the first, second, and third molars. These data indicate the presence of genetic factors regulating susceptibility to dental caries, confirming similar findings by Horowitz, Osborne, and DeGeorge (1958). Comparisons of the

TABLE 1. MEAN INTRAPAIR VARIANCE ANALYSES OF CARIES EXPERIENCE RATIOS (CER)* FOR ALL TEETH AND FOR FOUR QUADRANTS OF THE MOUTH (DZ; DIZYGOTIC, MZ; MONOZYGOTIC)

Group	Present Study					Horowitz, Osborne, DeGeorge (1958)			
	Zygosity	Number Pairs	Variance	F	P	Number Pairs	Variance	F	P
All Teeth	DZ	19	130	6.84	<.001	13	79	2.72	<.025
	MZ	19	19			22	29		
Ant. Max	DZ	19	152	4.22	<.005	17	59	1.59	>.10
	MZ	19	36			27	37		
Ant. Mand.	DZ	19	39.5	4.82	<.001	19	45.2	5.58	<.001
	MZ	19	8.2			30	8.1		
Post. Max.	DZ	19	290	6.04	<.001	17	248	2.64	>.01
	MZ	19	48			26	94		
Post. Mand.	DZ	19	221	5.02	<.001	15	156	2.48	.025
	MZ	19	44			25	63		
Active Caries	DZ	19	26.0	5.31	<.001				
	MZ	19	4.9						

$$*\text{CER} = \frac{\text{Number of decayed or filled surfaces}}{\text{Number of surfaces available for decay}} \times 100$$

present study with earlier twin studies of dental caries are meaningless because earlier studies used the whole tooth as the unit of measure whereas Horowitz *et al.* and the present authors have used the surface as the unit.

Corresponding data (variances rounded) from the study of Horowitz *et al.* are also included in Table 1. It is of interest to note that the closest correspondence between the two bodies of data is found in the variances calculated for the anterior mandibular quadrant. Though this similarity may be no more than coincidence, it may also reflect the well known fact that teeth in that quadrant are more resistant to decay than those in any other quadrant of the mouth. Their resistance is thought to be due to their anatomy (self-cleansing, absence of pits and fissures) and to their location within the mouth which leads to their being constantly bathed in the buffered, neutral to slightly alkaline S & S saliva.

A second point of interest is the fact that with one exception, the variance ratios calculated were higher in the present study than the corresponding ratios in the study of Horowitz *et al.* Ethnic and environmental differences between the two twin samples in addition to test and sampling errors may account in part for the differences noted. The agreement between findings on anterior mandibular teeth suggests that these sources of differences may not be major factors. A more likely explanation may be developed from the fact that the present twin series is a younger group. Twins in the study of Horowitz *et al.* ranged from 18 to 55 years of age with a median at 24 years. Only 2 pairs in the present series were over 24 years of age. The arithmetic bases for the larger variance ratios of the present study are that the variances for monozygotic twins in the younger series are either about the same as or considerably smaller than those of monozygotic twins in the older series. In addition, the variances for dizygotic twins in the present study are either about the same as or considerably larger than the corresponding data from Horowitz *et al.* In general, it might be anticipated that intrapair differences in caries experience of twin pairs would tend to increase with the increasing environmental diversity accompanying aging, at least up to the point where one member of a pair has at least one caries per surface. At the same time, if it is assumed that the intrapair correlation in ages at eruption of the teeth is higher in monozygotic than in dizygotic twins, then differences in length of exposure to risk of decay make a greater contribution to variability within dizygotic than within monozygotic pairs. However, intrapair differences in ages at eruption would tend to become less significant as twin pairs age, and would become small relative to the total length of exposure in twins over 30 years of age. Hatton (1955) reported that the intrapair variance in ages at eruption of deciduous teeth was higher in dizygotic than in monozygotic twins. Hence, the larger variance ratios of the present study may have resulted from the inclusion of intrapair variability in ages at eruption of teeth, a source which may have made an insignificant contribution to the total variability in the older series.

Data on active caries were included in all CER tabulations presented in Table 1, but, for reasons given below, were also considered apart from filled caries. The number of active caries i.e., carious lesions which have not been filled, depends on the number of surfaces available to decay, the caries attack rate, and the frequency and quality of dental care received. In addition, the number of active caries observed depends

on methods used to detect such caries. It had been anticipated that twins in the present study, particularly those in the University group, would have had better than average dental care, and, hence, that the frequency of active caries might not show significant heritability. Knutson and Klein (1938) suggested that tooth mortality, i.e., the number of teeth removed because of advanced caries, is a reasonable criterion of the level of dental health care. The fact that only 22 of the 76 individuals studied had lost any teeth due to advanced caries and that the average tooth mortality was 0.5 tooth per individual indicated that the twins in the present study had received superior dental care. In spite of a high level of care, there were only 3 persons found to be free of active caries and the average number of carious surfaces in the total sample was 8.4. The heritability suggested by the data on active caries (Table 1) may reflect the effects of both biological factors responsible for caries and the psychic and socio-economic factors which encourage (or discourage) frequent visits to the dentist. That we have measured behavioral as well as more purely physiological variation is suggested by the total correlation coefficient (.16), calculated from all individuals, between the number of active and the number of filled carious surfaces which did not differ significantly from zero.

Many investigators (see Neel and Schull, 1955) have pointed out some of the difficulties in interpreting the heritability estimates often accompanying twin studies. On the other hand, Clark (1956) pointed out that such estimates are of interest from the evolutionary point of view as indices of the susceptibility of characters to genetic change. The h^2 values in Table 2 were calculated from our data and from those of Horowitz *et al.* using the formula:

$$h^2 = \frac{V_{DZ} - V_{MZ}}{V_{DZ}},$$

in which h^2 is the proportion of variability in dizygotic twins attributable to genetic variation and V_{DZ} and V_{MZ} are the respective intrapair variances of dizygotic and monozygotic twin pairs. Since nearly all persons are edentulous at birth and many end life in the same condition, one would expect some age between eruption of the first tooth and loss of the last to yield a maximum h^2, and these data suggest that the maximum occurs no later than the mean age in the present series (18.7 years). Though differences in the h^2 values in the two studies are completely dependent on the variance differences already discussed above, they are included because they may be illustrating an age effect. The h^2 values for all traits considered are included without comment in Tables 3 and 4.

Microflora. A summary of computations based on logarithmic transformations of bacterial counts is presented in Table 3. Ever since Miller's series of classic papers (see Cox, 1952) on the pathogenesis of dental caries in which he assigned oral microflora a significant role as acid formers, investigators have sought to explain the relationship between species and numbers of microorganisms in the mouth and the frequency of caries. Both streptococci and lactobacilli have been suggested as important etiological agents in dental caries both on the basis of their acidogenic power and on the basis of the correlations observed between number of organisms and number of decayed, missing or filled teeth (e.g., Sullivan and Storvick, 1950 and

TABLE 2. HERITABILITY ESTIMATES (h^2) OF CARIES SUSCEPTIBILITY FROM PRESENT STUDY AND FROM HOROWITZ ET AL. (1958)

Group	$h^2 \times 100$	
	Present Study	Horowitz et al.
All Teeth	85	63
Ant. Max.	76	36
Ant. Mand.	79	82
Post. Max.	84	62
Post. Mand.	80	60

TABLE 3. MEAN INTRAPAIR VARIANCE ANALYSES OF CERTAIN ORAL MICROFLORA; BASED ON LOGARITHMIC TRANSFORMATIONS OF ORIGINAL COUNTS

Culture	Zygosity	Number Pairs	Variance	F	P	$h^2 \times 100$
Miscellaneous Bacteria	DZ	17	0.057	3.35	$<.01$	70
	MZ	17	0.017			
Streptococci	DZ	17	0.082	3.90	$<.01$	74
	MZ	17	0.021			
Lactobacilli	DZ	17	2.7	1.35	$>.25$	25
	MZ	17	2.0			

Jay, 1936). Whether a relationship exists between dental caries and number and kinds of oral microflora in twins in the present study will be discussed in a future paper. However, the data presented in Table 3 are of interest apart from their possible relationship to caries.

The obvious question which arises in connection with the heritability shown by both the miscellaneous organisms and streptococci is the degree of independence between the two populations. The correlation between the two groups of organisms was .62 ($P < .01$ if $\rho = 0$) suggesting that either the streptococci are contributing to the heritability shown by miscellaneous organisms or that both are correlated with a common factor or factors. The miscellaneous organisms were not subcultured for identification of species present so that we have no direct evidence on this point. Studies by Bibby, Volker, and Van Kesteren (1942) and Richardson and Jones (1958) indicated that streptococci were among the most numerous of all major groups of oral microflora.

The lactobacilli counts showed extreme variability (0 to 8.5 million) between individuals. Permar, Kitchin, and Robinson (1946) noted that even on replicate platings of the same specimen of saliva lactobacillus counts showed wide variations. The use of log transformations failed to yield a distribution approximating the normal. Until some method is devised for eliminating non-random count fluctuations, further attempts to determine the heritability of lactobacilli counts seem likely to fail. Of course, all of the variability may be due to environmental factors, but, if true, its non-random character still demands some explanation.

Rate of flow, pH, and amylase activity. The pertinent statistics regarding rate of flow, pH, and amylase activity are presented in Table 4. Rate of flow was based on

cc. of saliva secreted in 15 minutes, proportional adjustments being made for those specimens collected over a longer or shorter period of time. Analyses of rate of flow were based on logarithmic transformations of raw data because of the non-normal character of their distribution. If rate of flow is related to caries frequency as has been suggested by investigations on experimental animals and humans (see review by Cox, 1952), the heritability shown by rate of flow is in part responsible for the heritability shown by dental caries, and a small beginning has been made in isolating the genetic variables which contribute to the decay process.

Data from the secretions of the parotid are not independent of the corresponding data from S & S secretions. The lack of correspondence between findings from the two types of specimens, particularly with regard to pH determinations, is probably due to technical difficulties in the collection of S & S salivas. Parotid secretions were extremely unlikely to be contaminated by S & S saliva, but the converse was not always true. Specimens of S & S saliva known to be contaminated with parotid saliva were eliminated, but minimal contamination may well have gone undetected. Further, S & S secretions contain the oral microflora whereas no organisms were detected in parotid specimens plated out during preliminary work. The presence of small amounts of parotid saliva and of the oral microflora in S & S saliva would tend to lower the pH of these specimens.

A study of titratable alkalinity and acidity of whole saliva by Turner, Bell, Scribner, and Meyer (1953) is the only genetic study known to us which is directly related to any of the three variables considered in Table 4. They found that

TABLE 4. MEAN INTRAPAIR VARIANCE ANALYSES OF RATE OF FLOW, pH, AND AMYLASE ACTIVITY OF PAROTID AND SUBLINGUAL-SUBMAXILLARY (S & S) SALIVA SPECIMENS

	Characteristic	Zygosity	Number Pairs	Variance	F	P	$h^2 \times 100$
Parotid	Rate of Flow	DZ	18	0.217	4.82	<.005	79
		MZ	16	0.045			
	pH	DZ	16	0.284	6.60	<.001	85
		MZ	17	0.043			
	Amylase Activity	DZ	19	184	4.60	<.005	78
		MZ	19	40			
S & S	Rate of Flow	DZ	18	0.089	2.78	<.025	78
		MZ	17	0.032			
	pH	DZ	17	0.10	—	—	—
		MZ	17	0.13			
	Amylase Activity	DZ	18	10.82	12.30	<.001	92
		MZ	17	0.88			
Par.-S & S Amylase Activity Ratio		DZ	18	73	4.87	<.005	79
		MZ	17	15			
Whole Stim. Sal. pH		DZ	12	0.080	3.08	<.10	68
		MZ	12	0.026			

the variance of titratable acidity of salivas from 18 pairs of monozygotic twins was significantly smaller than the variance in 18 pairs of siblings. In the present study, the heritability shown for pH probably contributes to the total heritability in dental caries if the correlation coefficient of .196 found by Sullivan and Storvick (1950) between DMF (decayed, missing, filled) teeth and salivary pH described a causal relationship.

The explanation for the amylase activity of S & S salivas exhibiting higher heritability than corresponding data from parotid saliva is unknown to us. Random effects and the fact that the S & S activity of one dizygotic and two monozygotic pairs was not measured offer possible explanations. Further, the fact that in our subjects the amylase activity of parotid saliva was 5-fold higher on the average than S & S activity may be in part responsible. However, one would expect random errors to affect specimens with less activity more than those showing greater activity. Though the rate of flow, pH, and amylase activity of saliva appear to be appreciably influenced by genetic factors, the heritability shown by one or more of these variables may be spurious because the components are not independent of each other. It may be possible to compute corrected heritability estimates after the interrelationships between these three variables have been examined.

During preliminary work it had been noted that the ratio of parotid to S & S amylase activity appeared to be somewhat fixed for each individual regardless of the absolute differences which existed between specimens collected at different times of day or on different days. This impression was supported by a comparison computed for monozygotic and dizygotic twins as shown in Table 4. It is possible that the heritability of ratio differences suggested by these data may not be independent of the intrapair differences in either parotid or S & S amylase activity, i.e., that all three sets of differences reflect the same genetic background. However the correlation coefficients between intrapair ratio differences and parotid differences were $.33 \pm .24$ and $.02 \pm .25$ for dizygotic and monozygotic pairs, respectively, and between ratio differences and S & S differences $.34 \pm .24$ and $-.24 \pm .25$ for dizygotic and monozygotic pairs respectively. These coefficients suggest that whatever genetic factors may be involved in determining intrapair parotid–S & S ratio differences, they are not principally those affecting intrapair differences in either parotid or S & S amylase activity. Genetically influenced differences between the parotid and S & S glands in their relative size, their relative proportion of cells producing amylase, or in their response to nervous stimuli are obvious possible explanations for the heritability shown by parotid–S & S ratios.

DISCUSSION

Throughout the present paper, traits have been described as exhibiting "heritability" whenever the intrapair variance in dizygotic twins has significantly exceeded the intrapair variance in monozygotic twins. This definition of heritability is subject to certain reservations and may not always reflect purely genetic differences. It is entirely probable that parents may tend to treat monozygotic twins more nearly alike than dizygotic twins. The estimates of heritability are based on the assumption that the average intrapair differences in environment are the same for monozygotic

and dizygotic twin pairs. Any significant tendency for greater similarity of environment in monozygotic pairs as contrasted with dizygotic pairs would tend to increase the apparent significance of the heritability estimate.

The twin study method is also limited by the fact that we can gain no information concerning the number of gene pairs that may influence a particular trait. Similarly, this method will not permit separation of the effects of a gene that may influence more than a single trait. In this study, for example, salivary rate of flow, pH, and amylase activity have been treated as if they were distinct and independent traits in both parotid and S & S salivas. Analyses of the data indicate that each of these three variables in parotid saliva is correlated with the corresponding variable in S & S saliva, and further that all three variables are correlated with each other. We have no way of knowing whether these correlations represent multiple effects of a single gene, or interactions of multiple genes.

Finally, the genetic factors whose existence is indicated by the heritability estimates may have primary actions far removed from the attributes that have been measured. For example, if there are genes which influence dietary preference for carbohydrates, the high carbohydrate diet might in turn affect amylase activity.

In the light of the limitations and qualifications that must be placed on the twin study method in general, we are reluctant to consider the heritability estimates obtained in this study as more than crude approximations. However, twin studies have certain obvious advantages and can be particularly valuable when they serve as preludes to more rigorous genetic analyses.

SUMMARY

The purpose of the present study was to determine whether genetic factors influence the frequency of dental caries, the rate of flow, pH, and amylase activity of saliva, and the number of microorganisms in the mouth through a study of these attributes in like-sexed twin pairs. It has been shown that the mean intrapair variances of the CER (caries experience ratio) of dizygotic twins exceeded those of monozygotic twins whether one considered the entire mouth or the dentition by quadrants.

Oral microflora were cultured on three media, and significant heritability was calculated for miscellaneous organisms and for streptococci. The degree of independence between these two cultures is presently unknown. The influence of genetic factors on oral lactobacilli could not be determined because of non-random variations in lactobacilli counts.

Similarly, variance ratios computed for rate of flow, pH, and amylase activity of both parotid saliva and a mixture of sublingual and submaxillary saliva specimens suggested that genetic factors influence these variables to a significant extent. The only salivary component measured which did not show significant heritability was the pH of S & S saliva. This exception was probably the result of technical difficulties.

The generality of the findings on salivary components has yet to be confirmed. It appears that several salivary components thought to be related to caries frequency may be regulated to an appreciable extent by genetic factors, and hence, the heritability shown by dental caries may have been partitioned to some extent. The relationship between each of the salivary components studied and dental caries will

appear in a subsequent paper (Goodman, Luke, Rosen, and Hackel, in manuscript). Plans are being made to extend these studies to family groups.

ACKNOWLEDGMENTS

We wish to thank Dr. F. W. Wertheimer, Sr. for his advice and support, Drs. C. Nash Herndon and Philip J. Clark for their critical reading of the manuscript and for their valuable suggestions during the course of the study, and Mr. Raymond S. Corwin and Miss Aura Sears for technical assistance. A special debt is owed to the remarkably cooperative twin pairs who served as subjects for this study.

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Further Observations on the ABO Blood Groups and the Sex Ratio

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IN A RECENT REPORT on the ABO blood groups and the frequency of male offspring at birth, Shield, Kirk, and Jakobowicz (1958) presented two sizable samples—the Melbourne and Perth series—which provide a valuable contribution to the study of the blood groups and the sex ratio. It seems worthwhile to examine further their hypothesis, to comment on their reference to the "heterogeneity of samples" in the study of Cohen and Glass (1956), and to reconsider the possible relationship between the ABO blood groups and the sex ratio, a problem still to be resolved.

The hypothesis of Shield *et al.* "that incompatible anti-A or anti-B antibodies crossing the placenta into the fetus selectively eliminate more males than females in early uterine life" neither explains the aberrancies in sex ratio indicated by those authors themselves nor those pointed out by Cohen and Glass (1956). There is as yet no significant evidence for any association between ABO-incompatibility and aberrant sex ratios.

This is shown by a direct χ^2 analysis of the frequency of male offspring when pooled ABO-compatible combinations are compared with pooled ABO-incompatible mother-offspring combinations, in both the Melbourne and Perth samples (presented by Shield and coauthors in Table 2 of their paper) as well as in other samples (Table 1). In no series is there any significant difference in sex ratio between ABO-compatible and ABO-incompatible mother-offspring combinations, nor is there any consistent trend in pattern. In some series, the ABO-compatible combinations have a higher proportion of male offspring than the ABO-incompatible combinations; in others the ABO-incompatible combinations have the larger proportion of male offspring.

In the second place, if ABO-compatibility status were in fact associated with the sex-ratio of offspring in mother-offspring combinations, it would be expected that any specific combination having a significantly *low* sex ratio would be *opposite* in ABO-compatibility status to any specific combination with a significantly *high* sex ratio. Such is not the case, either in the Melbourne and Perth samples or in the Baltimore series samples. In the Melbourne and Perth samples, *both* of the mother-offspring combinations which deviate significantly in sex ratio (AB children of A mothers—a high male ratio; and A children of B mothers—a low male ratio) are *ABO-incompatible combinations* (i.e., are similar to each other in regard to ABO-compatibility status), yet they deviate in sex ratio in opposite directions from the sample mean (sex ratio). In the Baltimore Rh (nonsensitized) and Pooled B-S-J

Received October 17, 1958.

* Public Health Service Research Fellow of the National Heart Institute during part of this investigation.

TABLE 1. SEX RATIO IN OFFSPRING

Sample	ABO-Compatible Mother-Offspring Combinations		ABO-Incompatible Mother-Offspring Combinations	Comparison of Sex Ratio ABO-Compatible versus ABO-Incompatible Combinations	
				χ_1^2	P
	Percentage of Males				
Shield *et al.* 1958					
Melbourne	53.11	>	51.27	2.34	>.10
Perth	53.51	>	52.02	0.57	>.30
Sanghvi, 1951					
Bombay	55.62	>	51.70	1.564	>.20
New York	52.53	<	53.13	.022	>.80
Johnstone, 1954	51.36	<	54.13	1.183	>.20
Cohen and Glass, 1956, and unpub.					
Balto. Rh nonsensitized	50.89	<	51.59	.101	>.70
Balto. Rh sensitized	53.01	>	50.14	1.038	>.30
Balto. Paternity	50.13	>	49.74	.026	>.80
B-S-J	51.74	<	52.18	.123	>.70

B-S-J = Pooled Baltimore Rh-nonsensitized, Baltimore Paternity, Sanghvi (Bombay and New York Samples), Johnstone series.

Balto. Rh nonsensitized = nonsensitized to Rh
Balto. Rh sensitized = unpublished new series, sensitized to Rh
} Balto. Rh negative women, 90% white

Balto. Paternity = Rh mixed, mostly Rh positive, 75% Negro.

Shield *et al.* = Rh mixed series.

Sanghvi, Bombay = Rh unspecified, probably Rh positive entirely or mainly. New York = "mostly of Rh-negative mothers"

Johnstone = Rh mixed.

samples (Cohen and Glass, 1956), *both* of the two mother-offspring combinations that show aberrant sex ratios (B children of B mothers and A children of A mothers) are *ABO-compatible*, yet they deviate in respect to sex ratio in directions opposite to one another (B-B combinations show an aberrantly high sex-ratio; A-A, an aberrantly low sex ratio). These findings argue against any association between the sex ratio in offspring and ABO-compatibility of the mother-offspring combinations.

On the other hand, an hypothesis postulating preferential fertilization of specific types of ova by specific types of sperm, such as was proposed by Cohen and Glass (1956), still remains plausible on the basis of: (1) the actual numerical values of the human sex ratio [the high sex ratio among B offspring of B mothers and the low sex ratio among A offspring of A mothers observed in all samples (viz., Baltimore Rh, (nonsensitized), Baltimore Paternity, Sanghvi's New York and Bombay series, Johnstone's sample, and the Melbourne-Perth series)] and (2) experimental data and natural observations in human and other organisms [specific absorption of A and B antibodies by sperm (Landsteiner and Levine, 1926; Yamakami, 1926; Hirszfeld-1928; Gullbring, 1957); the presence of specific maternal antibodies in the reproductive tract (Brambell, 1951); inbred rodent lines of high sex ratio and low sex ratio postulated to be attributable to biochemical influences on selective fertilization involving metabolism of ova (King, 1918); similar high and low sex ratio lines of mice

postulated to be attributable to selective elimination of X or Y sperm (Weir, 1958); directed fertilization of ova by B-carrying sperm nuclei in maize (Roman, 1947); and other evidence].

It is still too soon to accept any hypothesis, because the existence of an association between the ABO blood groups and the sex ratio of offspring has not been unequivocally established. The statistical evidence based on concordant trends of widely diverse population samples does favor some association between the occurrence of aberrant sex ratios in the offspring and certain mother-offspring combinations of ABO blood groups (that is, low sex ratio among A offspring of A mothers and high sex ratio among B offspring of B mothers). In these fundamental respects, the Melbourne-Perth sample stands in agreement with our own (see Shields *et al.*, Table 4). As indicated above, in the Melbourne-Perth sample the sex ratio among A offspring of A mothers is below the sample mean and the sex ratio among B offspring of B mothers is above the sample mean; indeed, all samples reported so far involving non-isoimmunized mothers show these phenomena irrespective of the ABO or Rh distributions of the samples. On the other hand, more data of various kinds are necessary. Thus we agree with Shield and his coworkers concerning the necessity of acquiring larger samples that are *internally homogeneous*.

We must point out, however, that the inferences made by Shield *et al.* concerning multiple ascertainment in the Baltimore sample and heterogeneity within and between samples involve a misinterpretation of our procedures. Both single and multiple ascertainment were used in the Baltimore samples, and the results of each were presented separately. Furthermore, in order to avoid any possibility that multiple ascertainment per woman might "contribute fortuitously to an apparent heterogeneity in the number of male and female offspring in some of the mother-child combinations," as Shield *et al.* suggest, a comparison of the sex ratio in each of the 14 combinations obtained by single ascertainment with that of each of the combinations obtained by multiple ascertainment was made (i.e., a cell by cell χ^2 analysis). No significant difference was observed in the sex ratio obtained by single as against multiple ascertainment (Pooled Single versus Pooled Multiple). [$\chi^2_{14} = 8.510$; $p > .80$—a corrected value, erroneously given as $\chi^2_{14} = 0.67$ in the published paper.]

In respect to the pooling of very "dissimilar samples" (Sanghvi's, Johnstone's, and the Baltimore series), it must be noted that the samples were *not dissimilar in sex ratio*, as indicated by a cell-by-cell analysis [$\chi^2_{28} = 25.44$, $p > .50$, Cohen and Glass, 1956]. It is this absence of dissimilarity in *sex ratio* that justifies pooling the data for the *sex ratio* analysis. We were of course aware that Sanghvi's Bombay and New York samples differed in both ABO and Rh distributions [Bombay: almost entirely D-positive; New York: mostly D-negative], in addition to differences existing among the other samples in ABO and Rh distribution, the latter because of the mode of ascertainment (Baltimore Rh series). It was precisely for this reason that each sample was presented separately, as well as in pooled samples (Pooled Baltimore and Pooled B-S-J). In fact, it is all the more noteworthy that each sample, despite the heterogeneity *between* samples in blood group distribution, geographical origin, socio-economic background, etc., shows internally a *similar pattern* of aberrant *sex ratio* among the mother-offspring combinations A-A and B-B. Although, as Shield

et al. demonstrated, the number of samples gathered thus far (Sanghvi's Bombay and New York, Johnstone's London, the Baltimore samples, and the Melbourne and Perth sample) does not place these deviations outside the 5 per cent limit of probability, the consistency of the pattern in samples from such diverse populations does warrant further investigation. It will be at least helpful that future samples may be tested in the light of a definite hypothesis.

(Since this article was submitted, Allan (1959) has reviewed ten series published before 1950, as well as the more recent ones quoted here. From these observations he concludes: ". . . the sex ratio of the white O babies of O mothers is, in the aggregate, significantly higher than that of A babies of A mothers. . . . From these observations it may be concluded that the existence of blood group differences in the human sex ratio at birth is now a very strong probability, at any rate in the white races.")

It is therefore also important to emphasize the need for more and larger independent samples which, while internally homogeneous, differ from other samples in regard to blood group distribution (ABO, Rh, etc.), geographical and racial origin, socioeconomic status, etc. From numerous studies on the determination of sex ratio in man and other organisms (King, 1918; Gershenson, 1928; Sturtevant and Dobzhansky, 1936; Crew, 1937, 1954; Novitski, 1947; Shaw and Mohler, 1953; Novitski and Sandler, 1956; Sandler and Novitski, 1957; Weir, 1958; and others), the protean nature of the problem has become apparent. The human sex ratio at birth is doubtless the resultant of a most complex interaction of multiple determinants among which the ABO blood group combinations of ovum and sperm, or possibly mother and offspring, may be only one.

Until a comparable group of samples which casts doubt on the existence of an association between the ABO groups and the sex ratio becomes available, it is not justifiable to assert that the demonstrated association is absent. Such a body of data would have to be comparable in size, etc., to the Baltimore Pooled Multiple, Sanghvi's New York and Bombay, Johnstone's London, and the pooled Melbourne and Perth samples, and fail to show A offspring of A mothers with a sex ratio below the sample mean, or B offspring of B mothers with a sex ratio above the sample mean.

To establish an association between blood groups and sex ratio with any reasonable assurance will be no mean undertaking—for the samples (of the type specified above) must be analyzed to take account of maternal age, birth order, family size, season of birth and so on, in order to extract those other factors which influence sex of offspring. Only such a detailed statistical and epidemiological study of more and larger samples, with possible supplementary experimental evidence from laboratory studies on human material in vitro, can determine whether the ABO blood groups really do affect the sex ratio; and if so, in just what way and how it is brought about.

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BOOK REVIEW

Inborn Errors of Metabolism

By DAVID YI-YUNG HSIA. Chicago: The Year Book Publishers, Inc. 1959, pp. 358. $9.50.

DR. HSIA'S NEW BOOK is an interesting and welcome addition to the few reference books now available in the field of medical genetics. In a concise and lucid fashion Dr. Hsia has compiled pertinent genetic, clinical, and pathophysiological data on nearly one hundred conditions which he subsumes under the definition of "inborn errors of metabolism". Diagrams depicting the general phenomena of unitary genetic events leading to multiple phenotypic changes are included wherever possible and generally are very helpful. An appendix containing techniques pertinent to the hereditary diseases discussed is included, which together with the text makes for a unique reference book, useful not only to the clinician but to the research worker and teacher as well.

There are several specific points which the author might consider changing in future editions of this book. (1) On page 59, he states that ". . . the genes for thalassemia and sickle cell hemoglobin are located on different chromosomes." Actually, all we know about these markers today is that they are both autosomal and probably not allelic. (2) On page 114 under Alkaptonuria (Heredity) the 3rd sentence is clearly a nonsequitur which should be corrected. (3) References to detection of heterozygotes (phenylketonuria, galactosemia, cystic fibrosis, etc.) should be carefully qualified as to accuracy and sensitivity of detection ability.

Finally, Dr. Hsia's concept of inborn errors of metabolism and his subclassification of genetic diseases under this heading deserves comment. On page 27, last sentence, he states that the definition of inborn errors of metabolism should be extended to include ". . . any genetically determined condition where there is clear-cut evidence of a chemical disturbance, and even include those conditions which are likely in the future to have a chemical basis." Indeed, it would be difficult to conceive of any genetic disturbance which did not manifest itself through chemical-physical pathways. Such an all inclusive definition has little value except insofar as it would permit the author to cover the entire field of medical genetics if he so desired. Fortunately, Dr. Hsia has not been excessive in the application of his definition and the majority of the material covered retains a considerable degree of homogeneity.

Dr. Hsia has then attempted to subclassify the inborn errors of metabolism according to the nature of the primary genetic effect involved. He has grouped these conditions into four classes: (1) disturbances in molecular structure, (2) disturbances in molecular synthesis, (3) disturbances in molecular function, and (4) disturbances in renal transport mechanisms. Disturbances in function result from structural changes or deficiencies, and should not be considered primary genetic effects in themselves, while the renal transport category is clearly out of place in terms of primary genetic effects. Actually, a classification in terms of primary genetic effects would consist of only two categories: (1) where the mutation had resulted in the formation of a new molecule, and (2) where the mutation had resulted in the absence of formation of any kind of molecule. Since the information available to us today would permit classification of only a handful of conditions in this way, such taxonomies seem

impracticable. Fortunately, Dr. Hsia has used his scheme only in a very broad sense and within his classes has grouped the various diseases according to good phenotypic criteria.

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Population Studies: Animal Ecology and Demography

Cold Spring Harbor Symposia on Quantitative Biology. Vol. 22. Cold Spring Harbor, New York: The Biological Laboratory, 1957. $8.00.

IT IS NOT TOO SURPRISING that human and animal demography or ecology have evolved in different and almost divergent directions, if one considers how different their aims and their objects of study happen to be. However, it was good that these two disciplines were offered an opportunity to meet at the 22nd Cold Spring Harbor Symposium.

It is difficult to say how far the Symposium has in practice permitted flirtation between the two demographies which was advocated by the Chairman of the first session, and it is also out of the purpose of this review to analyse some concepts which have formed the centre of most discussions, e.g. "density-dependent" and "density-controlling factors". Ecologists are there faced by the almost hopeless task of disentangling complex causal relationships, usually without the benefit of proper experimental set-ups and simply relying on the observation of natural events, a situation which lends itself easily to endless discussion. The readers of this journal will be interested mostly in that fraction of the Symposium which deals directly or indirectly with human affairs and especially genetics which, although not appearing in the title of the Symposium, has nevertheless, formed the subject of one session and might profitably have formed more of it.

Among the more strictly genetical papers of the Symposium, was one by Dobzhansky, who added a new and interesting demonstration in *Drosophila* of one particular way in which genetic drift may disguise itself, and which might have some bearing on the distribution of blood group frequencies. A paper of Sutter and Tran-Ngoc-Toan gave first results on the analysis of the distribution of distances between places of birth of spouses, showing that like any other investigated organism, man has ways of gene diffusion which are most unlike those of simple molecules. This is perhaps not surprising, but might be embarassing, in consideration of the fact that most present theories of population in Genetics are based on such an assumption.

Braidwood and Reed have summarised some evidence on the levels of population density in various areas during late pre-historic and early historic times. So scanty is the evidence and so interesting the issue that the inevitable conclusion is that it would be of the greatest importance to enlarge our very limited demographic knowledge of primitive people living to-day, while they are still primitive.

Birdsell has reconstructed with pencil and paper the possible time which may have been necessary for Negritos to colonise Australia. This reconstruction is based on the assumption that the population doubles at every generation and that newly founded colonies will begin to "bud" once they have reached a critical stage. The assumption of doubling is based on actual observations but very few data are available to suggest critical sizes at which "budding-off" may take place or the size of the "buds" which leave the tribe to settle somewhere

else. Using a wide range of plausible assumptions however, the actual time taken to colonise Australia turns out to be remarkably low.

"Cultural" factors which have affected human evolution have been reviewed broadly by Hulse, whose analysis suggests that not enough emphasis is usually laid on the importance of inter-group selection. In fact, only intra-group selection is as a rule considered in standard population genetics, and an attempt to include inter-group selection might lead to one possible way of bringing together the Volterra-Gause-Lotka and the Fisher-Haldane-Wright approaches to evolution.

Among various reviews on present work in human demography, human geneticists may be particularly interested by a paper on sex ratio (B. Colombo) and an essay on the analysis of fertility (L. Henry). This last paper suggests that data from times when birth control was not practised and for which are available, occasionally, excellent records, may supply material for a study of the genetics of human fertility unaccompanied by psychological and social factors.

Although this was not its main aim, the Symposium itself therefore provides the human geneticists with some material for thinking and planning. It is to be hoped that sometime the initiative of favouring flirtation between human demography and genetics will take a more definite shape than was possible here.

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The Population Ahead

ROY G. FRANCIS, editor. University of Minnesota Press, 1958, pp. 160 + x. $3.75.

IN 1948 there was a population conference held at the University of Minnesota. This book is the report of a second conference, 10 years later. The authors represented are Pascal K. Whelpton, Ancel Keys, Frederick Osborn, Jan O. Broek, E. S. Deevey, Karl Sax, Robert Spencer, and Arnold C. Harberger, with comments by Warren Thompson, Philip Raup, Sheldon Reed, Alex Weingrod, and Dwight Minnich.

Most of the material is already familiar, but it is still impressive to read such statements as this one by Whelpton: "If the rate of growth of the last 30 years—about 1.2 per cent which also seems small—had started at the beginning of the Christian Era, we would now have more than 3000 people per square foot of the world's land area. And if the rate of growth of the last five years—which is only about 1.5 per cent per year—had been in effect we would now have more than a million people per square foot of land." The book is devoted to various aspects of this "explosion".

To mention only a few points: Frederick Osborn argues that it is impractical to discuss what the maximum or optimum total population should be, but that we can intelligently discuss what the optimum or maximum *rate* of increase might be. Broek calls attention to the fact that the world's capacity to consume other things grows far faster than its need for food, great as the food problem is. Edward Deevey takes a look at the human population through the eyes of an animal ecologist; the result makes for a pleasant intellectual exercise. Warren Thompson contrasts 1958 with 1948 predictions; among the surprises are the spectacular drop in death rates in many non-industrial countries, largely the consequence of insecticides and antibiotics, and the equally spectacular drop in birth rate in Japan, almost

50% in 10 years. He also notes that the two most populous countries now officially "recognize" the population problem. The two geneticists on the program, Karl Sax and Sheldon Reed, discuss points that certainly are not new to readers of this journal, the effects of a decreased intensity of selective mortality, and the possible dangers of an enhanced mutation rate.

The book is saved from being simply a heterogeneous collection of essays by the work of the editor Roy E. Francis, who has summarized and coordinated them most effectively.

Although most of the facts and ideas in the book are familiar, the problem it deals with is perhaps our greatest, provided we avoid destruction by atomic war. It merits the thoughtful consideration of everyone who thinks of the future.

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Radiographic Atlas of Skeletal Development of the Hand and Wrist

By W. W. GREULICH AND S. I. PYLE. Stanford University Press, 1959. Second Edition. 256 + xvi pages. $15.00.

IN 1950, W. W. Greulich and S. I. Pyle published their *Radiographic Atlas of Skeletal Development of the Hand and Wrist* which immediately became a standard reference, supplanting (in most radiographic installations) the earlier Atlas by Todd (1937) and Flory's 1936 monograph. Now Greulich and Pyle have completed a revised and enlarged edition of the Atlas, characterized by more contrast in the radiographic reproductions, several additional age standards, newly redrawn pictures of individual maturity indicators, a discussion of subjective error in hand-age assessment and the heaviest coated stock outside of art reproduction.

The Greulich-Pyle method, like Todd's from which it derives, reaches beyond simple counting of centers or planimeter measurement of areas, and averages the age-equivalents of individual bones and their epiphyses. Being keyed to a series of pictorial standards, the accuracy of individual determinations depends in part on the quality of the reproductions and on internal consistency within the original radiographs used as standards. From time to time various criticisms have been leveled against the Greulich-Pyle method, among them a series of papers by Donald Mainland. Greulich and Pyle devote a number of pages to a refutation, though they omit reference to potentially-competitive systems such as the "Oxford" method. Actually, reliability of the Greulich-Pyle method is usefully high if the work is done carefully and by a trained rater: replicability values for preadolescents approximate 0.98 in our laboratory. Equally pertinent to the technique is the question of secular trends in respect to skeletal age. The radiographs upon which the Greulich-Pyle norms are based were made, on the average, twenty years ago. We have evidence, in our Fels population, of a trend toward advancing bone age, thus these standards are relative rather than absolute.

Familial and presumably gene-determined differences in the patterning of hand ossification complicates skeletal-age assessment more when a simple carpal count is used than with the Greulich-Pyle method. The authors, though rightly minimizing the influence of most aberrant patterns on their system, unfortunately minimize the existence of the patterns. The pseudo-epiphysis on Metacarpal II, and the atypical late appearance of the Navicular, and greater and lesser Multangular do cluster in kindreds, and it is not safe to assume that the latter three never result in spuriously low age assessments.

In addition to the improvements mentioned, which include the revised terminology (N.T.)

for the carpals, there are numerous new tables, including those of Bayley and Pinneau for use in stature prediction. Besides, this new edition contains drawings for a lead-shielded tepee to minimize stray radiation during hand radiography. Granted its value in conjunction with obsolete, coneless desk-type machines, modern radiographic equipment with a proper rectangular double-diaphragm cone and lead rubber on the table would be better. Moreover, use of the 100 MAS technique with non-screen film, recommended in the Bayley-Pinneau section (p. 234) is not desirable when satisfactory films can be obtained at 10 MAs using screen-type film.

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Zur Kenntnis des "Mongolismus"

By HANNES W. KALB. Munich: Heilkunst-Verlag, Acta anthropol. No. 1 1957, pp. 66, DM 5.50.

THIS SMALL MONOGRAPH on mongolism, subtitled "a contribution to the anthropology of mental deficiency," represents the first of a series of publications planned by the Anthropological University Institute in Munich. It is organized in such a way that a detailed review of the literature, unfortunately somewhat outdated, is followed by the author's own data on 201 German mongoloids born between 1925 and 1955, and a lengthy discussion of the place of mongolism in both ontogenetic and philogenetic development.

Among the modern studies disregarded in the first section (Genetic Theories and Twin Studies) are the comprehensive data of Penrose (Lancet, 1954), Øster (Danish Med. Bull., 1956) and Allen & Baroff (Acta Genet., 1956). It is a matter of speculation, therefore, whether the author would want to be on record to the effect that "any hereditary factor can in no wise be ascertained" (p. 41) had he been familiar with the results of the recent investigations. Another statement which does not seem to be substantiated is that "children damaged by erythroblastosis fetalis owe their illness by no means to a hereditary mechanism but to a lack of prophylaxis" (p. 41).

By and large the findings regarding maternal age, birth order, and an increased incidence of abortions agree with those reported by other investigators. The social stratification of mongoloids was found to be similar to that of the general population. The author shares the current view that there is a reproductive deficiency in the mothers of mongoloids, but his conclusion that this defect is temporary in nature would seem to be arbitrary. His observation of a high incidence of chronic infections, "latent tetany" and various complications during pregnancy (including fright and grief) are difficult to evaluate without adequate controls.

As far as the etiology of mongolism is concerned, preference is given to the somewhat esoteric "fetalization theory," which at this time does not lend itself to experimental verification.

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Editor's note: This review was written before the data on chromosomal aberrations in mongolism were available.

Cytodifferentiation

Edited by D. RUDNICK. University of Chicago Press (1958), $3.75.

THE YEAR 1956 was marked by a series of conferences devoted to problems of developmental biology. "Cytodifferentiation" consists of an account of the proceedings of the conference, held at Brown University in July, 1956, which was devoted to that topic. It is one of ten volumes which record the content of the series. The organizers of the conferences (P. Weiss, general chairman) evidently realized that an unabridged account of all of the proceedings would have resulted in an extremely bulky and prohibitively expensive set of volumes. To avoid this, editors were assigned the task of providing condensed versions of the proceedings. Taped recordings, as well as notes taken by the editor, served as the basis for the volume. Each participant was asked to check statements attributed to him or to provide the condensed statement.

Dr. Rudnick, editor of this 131 page book, has done an admirable job of preparing a readable and lucid account of the cytodifferentiation conference. Though markedly abridged the text is not telegraphic, is easy to follow and is presented in a manner which retains some of the spirit of the interchange of ideas which took place during the discussions.

The topics covered were: "Cytodifferentiation and genic endowment", "Chemical and morphological indices of cytodifferentiation", "Special instances of cytodifferentiation: myogenesis, fibrogenesis", "The internal milieu of cytodifferentiation" and "Cancer, growth and differentiation". Each chapter has a summary and the volume is concluded with a chapter on "evaluation and perspectives". The list of participants is too lengthy to be repeated here, but consists of a distinguished array of investigators.

Ordinarily a verbatim account of a symposium contains papers which vary in quality. To a great extent this sort of variability has been eliminated in this case; some of the material is more exciting than the rest, but the manner of presentation is uniformly good. This is a volume of concentrated "meat". It is surprisingly up-to-date, considering the two years which have elapsed since the conference took place. Some of this is no doubt due (as evidenced by citations of papers which appeared after 1956) to author's revisions, some to the fact that later developments have not out-dated the discussions. In any event, this is a valuable volume which should be read by all students of developmental biology. As one who believes that the proceedings of important conferences should be accessible to all, I approve of the procedure which has made this volume available at a relatively modest cost.

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* Supported in part by research grant C-3874 from the National Cancer Institute, Public Health Service.

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